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Public Health

The order in this section is:—**General; Vital Statistics; Epidemiology; Maternal and Child Health; Health of Students; Nurses and Hospital Staffs; Health of Old Persons and the Chronically Ill; Chronic Cardio-Respiratory Diseases; Chronic Rheumatism; Cancer; Miscellaneous Diseases; Dental Health; Mental Health.**

VITAL STATISTICS

McMAHAN, C. A. **The Lower Limit of the Infant Mortality Rate in the United States.** *Milbank Memorial Fund Quarterly.* 1959, Oct., v. 37, No. 4, 337–46, 2 figs. [Refs. in footnotes.]

The infant mortality in the United States in 1956 was 26 per 1,000 live births and it was only 23·2 for the white population. The author considers the methods for estimating the irreducible minimum infant mortality and suggests that this lies between 7·6 and 12·3 per 1,000 live births. The lower of these limits was reached on the assumptions that the very small premature babies will continue to die at the same rate, that babies weighing 1,000 gm. or less (2 lb. 3 oz.) will contribute 4·1, congenital malformations 2·7 and violence 0·86 to the infant mortality. The upper limit was based on the assumptions that the babies under 1,000 gm. in weight will contribute a rate of 4·1, that a reduction of 52% in the number of deaths of babies weighing 1,000–2,500 gm. can be achieved and their contribution to the infant mortality can be reduced to 4·2, that 45% of the deaths of babies of over 2,500 gm. are preventable and their death rate would fall to 4·0, and that no deaths would occur in children surviving 28 days.

[These estimates of the minimum infant mortality are, probably, over-optimistic since in England and Wales a third of the infant deaths occur during the first day of life and another third during days 1–6. The death rate in the first few days of life has only declined very slightly during the past 50 years and a very large improvement would be necessary if the infant mortality is to be halved.] *W. J. Martin*

EPIDEMIOLOGY

YALE J. BIOL. & MED. 1959, Sept., v. 32, No. 1, 1–61, 12 text figs. & 5 figs. on 5 pls. [Numerous refs.] **Symposium: Perspectives in Epidemiology.**

This is a very interesting and exceptionally well presented symposium demonstrating that epidemiology is not confined to infectious disease, but is the basis of all preventive medicine and valuable in the investigation of diseases of obscure aetiology. The first paper (A.M.-M. PAYNE, *Global epidemiology today*, pp. 4–15) is an excellent review of world-wide epidemiology as it can be conducted by the World Health Organization. Man-made changes in the ecology of a disease, for example the use of insecticides in malaria control, must be made cautiously lest they create greater problems than they solve. The history of influenza A is very well reviewed, especially the apparent similarity of the virus causing a pandemic in 1889 and the Asian virus of 1957–8. Both these pandemics apparently began in China and it is suggested that the virus may have persisted in a lower animal such as the pig. The pattern of the evolution of poliomyelitis in modern times due to changing patterns of hygiene is reviewed: other enteric viruses such as Coxsackie B may follow a similar course and become much more important as more people reach adult life without being infected. WHO has recently designed a world-wide collection of sera according to statistically sound sampling methods. This collection is to be stored over a long period for studies of infectious diseases, blood groups, nutrition, etc.

Because of the complexity of their life history, knowledge of the arboviruses is more obscure than of the other main virus families (R. M. TAYLOR, *Studies on the ecology of arthropod-borne (arbor) viruses*, pp. 16–22).

In New England, eastern equine encephalomyelitis virus causes costly epidemics in horses and pheasants and occasionally severe encephalitis in man. Its presence has however been demonstrated over wide areas of South America and its distribution parallels that of the fly-ways of migrating birds. The virus does not appear to winter in the north in mosquitoes and it may be repeatedly introduced by birds. A beetle (*Tenebrio molitor*) has been found infectable by feeding and birds may be infected by eating these. In the search for eastern equine virus, a new virus (Hilo virus) has been isolated from a nestling bird. Studies are nearly complete of *Phlebotomus* fever virus and tick-borne virus strains from Egypt and the Sudan. There appear to be 4 or 5 new tick-borne viruses including 2 from *Argas* ticks. Groups A, B and C of arborviruses have been found cytopathic to different combinations of chick embryo, Pekin duck kidney and rhesus monkey kidney tissue cultures.

The third paper (F. R. FEKETY and I. L. BENNETT, Jr., *The epidemiological virulence of staphylococci*, pp. 23–32) discusses the recent appearance of unusually virulent strains of staphylococcus, particularly in hospitals. These are mainly of the type 80/81: for example 51% of 498 strains from clinical infections were of this type. It has a greater tendency than others to cause disease in infants and young children, but also quite commonly in adults. It persists longer in carriers and is the predominant cause of hospital cross-infections: it caused 75% of a series of clinical infections with onset after admission to hospital. The causes of the increased incidence of hospital infections are discussed.

The fourth paper (R. H. GREEN, *Asian influenza in New Haven, 1957–1958*, pp. 33–43) presents a study of influenza antibodies in 286 pairs of sera collected from individuals before and after an epidemic of Asian influenza. None of the first samples contained haemagglutinin-inhibiting antibody to Asian influenza, but 40% had complement-fixing antibody indicating previous infection with other strains of influenza A. About half the study population received Asian influenza vaccine during the outbreak. There was little difference in antibody response between the vaccinated and unvaccinated in any age group, but all except adults showed consistently less responses in vaccinated than unvaccinated groups. In comparison with the case histories, about 80% of those who thought they had had influenza appeared to be right and about 64% of those who thought they had not, appeared to be wrong. No differences were found attributable to social class or size of family.

In the United States measles is now causing more deaths than poliomyelitis (F. L. BLACK, *Serological epidemiology in measles*, pp. 44–50). Antibodies are always absent before onset of the rash, but appear within about 48 hours and appear to persist for long periods. In a sample of 300 sera from the New Haven area the antibody incidence rose from about 20% in preschool children to about 95% at 8–9 years of age. No difference in incidence by social class was found, but the incidence in children under 11 years of age was higher in large families. Inapparent or unrecognized infections following gamma globulin have been demonstrated during an institutional outbreak.

The sixth paper (T. H. INGALLS, *Congenital malformations and environmental influences*, pp. 51–57) discusses the elucidation of the aetiology of congenital malformations notably those attributable to rubella: congenital cataract where the infection was at the fifth week of pregnancy, congenital heart disease when it was at the sixth and seventh, and deafness at around the eighth week. Hypoxia in pregnant mice around the 8th day of pregnancy caused anencephaly, on the 14th day it caused cleft palate and on the 16th day "open eye". Exceptional cold during metamorphosis apparently caused a high incidence of six-legged frogs. Study of environmental factors in pregnancy may be expected to elucidate the aetiology of more human congenital defects.

The last paper (C. WHITE and H. EISENBERG, *ABO blood groups and cancer of the stomach*, pp. 58–61) discusses the evidence that certain blood groups are related to the occurrence of carcinoma of the stomach at certain sites. Thus, in males, group A is significantly commoner than group O in patients with carcinoma of the pylorus and antrum. *C. E. Gordon Smith*

MOSS, B. J. L. **Morbidity amongst the Staff of a Local Authority.** *Med. Officer.* 1959, Dec. 4, v. 102, No. 23, 293–5, 2 figs.

The 1,196 employees of the Metropolitan Borough of Bermondsey lost 15,991 days through sickness during 1958. The average number of days lost was 13·45 and this was contributed by 781 persons who had 953 spells of sickness. The data have been analysed by 9 departments, not differentiating sex, for the main causes of sickness absence. Almost two thirds of the employees were in 2 departments which consisted of skilled and unskilled manual workers. The percentage reporting sick ranged from 25 to 91 in the various departments. Bronchitis was the chief single cause of sickness accounting for 1/5th of all the days lost and 1/9th of all spells of sickness; the average time lost from this cause was one month. Upper respiratory infections were low; only 13% of the spells of sickness and only 4% of the days lost were due to this cause which had an average duration of 4·9 days. 119 persons contracted influenza and they lost on the average 13·5 days per attack.

[This paper illustrates some of the difficulties encountered in morbidity statistics. Many conditions which render one type of worker unable to work would not do so to another type. Thus enteritis only affected the manual workers; these had attacks of not more than 2 days' duration, but it was obvious that an attack of diarrhoea would be much more inconvenient to outdoor workers than to the office staff. Rheumatism was highest among manual workers who had an attack rate of 12% compared with 2% for other workers. The author suggests that those of a lower mental calibre, of whom many undertake manual work, have a lower threshold of pain but it is also just as likely that it is the nature of the job that determines whether the afflicted person can work or not. The term average annual duration in this paper does not mean the average length of sickness but the average number of days lost per person among the total population.] *W. J. Martin*

ROSENBAUM, S. **Cross-Sectional Review of Morbidity among Young Soldiers.** *Brit. J. Preventive & Social Med.* 1959, July, v. 13, No. 3, 103–27, 6 figs.

That this well documented study of morbidity in young soldiers from 1950–56 is the first article in this number of the journal to be produced by the new editors is in itself an index of its importance. At this time conditions of National Service and in the structure of the Army were relatively stable. Since 80% of the fittest males in the country were called up for National Service of whom two thirds served in the Army, this review can be said to reflect the health of the nation.

Between the beginning and the end of the 7-year period there is evidence of decided improvements. Geographical and climatic differences in the places in which they served were found to have a more marked influence on their health than the differences from year to year. In those men whose service had been deferred, a healthy home environment probably accounted for the improvement gained by a later call-up. In spite of the authorities' concern for every detail of the soldier's existence, respiratory diseases predominated in the U.K., intestinal diseases in the Middle East and skin diseases in the Far East. The recruit was especially liable to respiratory disease; otherwise, in the U.K. length of service was a more important factor than age in determining morbidity. A similar factor operated abroad when the soldier was exposed to new environment on arrival in a tropical command. *W. R. M. Drew*

MATERNAL AND CHILD HEALTH

MORLEY, D. **A Pattern for the Development of Child Welfare in Developing Countries.** *Mother & Child.* 1959, Oct., v. 30, No. 7, 163–7, 1 chart & 2 figs.

The author is a medical missionary working (with a Government grant) in Western Nigeria. He contrasts the English village, where doctors and nurses are at hand, and the child mortality is about 25 per 1,000, with the African village, mostly still without western medical facilities, and where the child mortality rate is about 500. The African child has multiple diseases—malnutrition with pneumonia or diarrhoea with malaria. The 3 diseases, diarrhoea, pneumonia and malnutrition, account for one-third of the deaths, and malaria, whooping cough and measles are serious.

The mothers readily bring their children (300–400 each day) to the clinic, where they are seen first by the 5 locally trained nurses and if necessary by doctors, and the opportunity is taken to vaccinate them against smallpox (which is still endemic), to give prophylactic pyrimethamine, and to vaccinate against whooping cough and tetanus if feasible. If tuberculosis is diagnosed, isoniazid is given, but BCG prophylaxis has not yet been found possible on a large scale.

"This welfare clinic has now passed out of the experimental stage and is considered an integral part of the hospital's work. Those who run this clinic believe that work along these lines will play some part in reducing the toll of death amongst babies and small children in this area." *Charles Wilcocks*

CHRONIC CARDIO-RESPIRATORY DISEASES

FLETCHER, C. M. **Chronic Bronchitis. Its Prevalence, Nature, and Pathogenesis.** *Amer. Rev. Respiratory Dis.* 1959, Oct., v. 80, No. 4, Pt. 1, 483–94, 7 figs. [41 refs.]

In this Robert Keeton Memorial Lecture given by a distinguished British authority on the subject, particular attention is paid to the apparent difference between the frequency and severity of the disease in Great Britain and the United States; according to the statistics bronchitis accounts for nearly 10% of deaths in England and Wales, and the death rate is 45 times greater than in the United States. Although the statistics are not comparable the difference between the morbidity rates of 2 countries seems to be very considerably less. American bronchitis shows no rise in prevalence in late middle age. The rate is greater for females than males, and the reverse occurs in England; the word "bronchitis" appears to refer to different things in the 2 countries. In all probability there is less difference in the incidence of cases, but it is probable that the disease is less disabling in later life and it is almost certainly fatal less frequently than in Great Britain.

Bronchitis has always been a major problem in Great Britain. Mortality from this cause declined in men aged 45–64 in 1920–1930, but this trend has been arrested, and there has been no fall with the advent of chemotherapy. It is possible that the effects of modern therapy are being masked by an increase in incidence due to cigarette smoking. It is only in the last few years that any attention has been paid to chronic bronchitis in medical literature. This may be due to the fact that morbidity and mortality from chronic bronchitis have always been borne by the poorer section of our community. In the United States, however, bronchitis mortality remains low and constant but there is a marked increase in the deaths registered as due to emphysema.

After a short clinical account of chronic bronchitis as it usually occurs in England, Fletcher went on to refer to the confusion caused by the differences in terminology. This, he thought, was due to failure to distinguish the essential features of the disease from its

complications. He suggests that the presence of cough with expectoration, persistent or recurrent, if there is no other cause than bronchitis, is sufficient to justify a diagnosis of chronic bronchitis. The inclusion by most authors of breathlessness as an essential symptom leads to confusion. In London postmen it was found that those producing 2 cc. or more of sputum in the morning had a reduction in ventilatory capacity. If this definition is accepted 2 associated types of disability must be considered. The first is liability to recurrent chest illnesses, and the second is dyspnoea. Based on this definition the author gives a suggested classification of chronic bronchitis. He considers that emphysema should be regarded as a morbid anatomical term, and not used as a clinical diagnosis unless the clinical, radiological, and physiological evidence is such that the presence of the morbid anatomical changes of emphysema can confidently be asserted to be present. He thinks that overactivity and hypertrophy of the mucous glands and goblet cells are the essential feature in chronic bronchitis.

In pathogenesis the only certain feature is that it is complicated and many different factors are involved. In Great Britain the striking factors are: (1) that the male/female sex ratio is about 4 to 1; (2) that the gradient of mortality rises steeply with declining social class; and (3) that atmospheric pollution must play a part, although the standardized mortality in rural areas is only reduced to one half of that of the whole country, and this cannot, therefore be the only factor. Many recent investigations have shown a close correlation between bronchitis and tobacco smoking. Investigation in general practice in Birmingham suggests that the social class gradient in the prevalence of bronchitis only appeared in smokers. For some reason or another it appears likely that the effects of smoking in producing bronchitis are less disabling in women than men.

None of these factors is sufficient to explain, however, the occurrence of chronic bronchitis in Great Britain; individual susceptibility must therefore be important in its pathogenesis.

[This is an excellent up to date review of the condition.] *F. H. Young*

VALENTIN, H., VENRATH, H. & KANN, J. Über die Bedeutung von chronischer Bronchitis und Lungenemphysem in der Arbeitsmedizin und die Häufigkeit dieser Krankheitsbilder bei mehr als 1000 Gutachten-Patienten. [**The Importance of Chronic Bronchitis and Emphysema in Industrial Medicine and their Frequency in over 1,000 Patients**] *Arch. f. Gewerbepath u. Gewerbehyg.* 1959, v. 17, No. 4, 420–29. [Numerous refs.]

Most of this paper consists of a not very illuminating account of chronic bronchitis and emphysema. The prevalence of these conditions in 1,031 patients referred to the Cologne University Medical Clinic is given. The patients were aged 40–60 years and had not worked in particularly dusty occupations. The figures illustrate the well known facts that chronic bronchitis and emphysema are common in such groups and that their prevalence increases with age. The paper does, however, include a useful list of German references on the subject. *I. T. T. Higgins*

GLYNN, A. A. **Antibodies to *Haemophilus influenzae* in Chronic Bronchitis.** *Brit. Med. J.* 1959, Nov. 7, 911–14, 1 chart. [15 refs.]

This antibody survey was undertaken to obtain more information about the relationship of *H. influenzae* to chronic bronchitis and particularly to acute exacerbations of this disease and to asthma.

Because of the antigenic heterogeneity of respiratory strains of *H. influenzae* an extract was made by a modification of the method described by TUNEVALL [this *Bulletin*, 1954, v. 29, 668]. This contained species-specific antigens common to all strains of *H. influenzae* and was used to coat tanned sheep red cells. Antibodies were estimated by the agglutination of these sensitized cells.

Sera were examined from 90 patients with chronic bronchitis and 33 with asthma and from 50 controls with non-respiratory disease. The age-distribution of the controls was similar to that of patients with bronchitis. The asthmatics had a younger average age than the other 2 groups. The sex distribution was approximately the same at all ages. 66% of patients with chronic bronchitis (defined as chronic cough and breathlessness for at least 2 years with acute infective episodes) had high titres of antibodies to *H. influenzae*: 1/500 or over. 21% of the asthmatic group and 6% of the control group had titres over 1/500 but the high antibody levels found in chronic bronchitis were not reached. No correlation could be found between antibody titre, age and sex of the patient, or the duration of disease. There was no constant association between the severity of the disease and the antibody titre, and except in one instance no significant difference between the titres of acute and convalescent sera from 14 patients with chronic bronchitis who had an acute infective episode.

The author concludes that his findings confirm the evidence relating *H. influenzae* to chronic bronchitis but do not support the theory that acute exacerbations of this disease are due to *H. influenzae*. *R. J. Fallon*

CANCER

DEAN, G. **Lung Cancer among White South Africans.** *Brit. Med. J.* 1959, Oct. 31, 852–7. [14 refs.]

Death certificates were examined for all white persons dying in South Africa between the years 1947 and 1956, whose causes of death were attributed to cancer of the lung (International List code numbers 162 and 163). A few certificates (2%) were eliminated because the cancer was specified as being respiratory cancer other than bronchial carcinoma or because of errors in coding. In 88% of the remainder the diagnosis had been verified by bronchoscopy and biopsy, thoracotomy or necropsy; further inquiries were made about doubtful cases.

Between 1947–8 and 1955–6 the male mortality increased from 117 to 238 per million living and the female mortality increased from 45 to 58 per million. The greater increase in the mortality among men is considered to indicate that the major part of the increase reflected a true increase in incidence.

Population counts were obtained from the Census Bureau for 1951 by age and sex for Union-born, British immigrant and other immigrant populations for each of the 5 largest cities, for all other urban areas and for rural districts. The 1951 data were regarded as providing estimates of the mean populations for the period 1947–56 and mortality rates calculated on this basis. At ages 45–64 years the mortality among male British immigrants was found to be higher than that of Union-born men in each of the areas studied and the total mortality among British immigrants was 44% higher than that expected from the age specific death rates for Union-born men in the relevant residential areas. In contrast, there was little difference between the observed and expected mortality among British immigrants at ages 65 years and over or among other immigrants in either age group. The mortality among male British immigrants was similar to—but, at most ages, slightly lower than—that recorded over the same period in England and Wales. The total South African male mortality was substantially lower than the British mortality.

The differences between South African and British mortality and that between Union-born and British immigrants can hardly be attributed to differences in cigarette consumption, since the white population of South Africa has been amongst the heaviest consumers of cigarettes in the world. In 1920 the South African white population consumed 43% more packeted cigarettes per annum than the population of the U.K.; in 1950 they consumed 40% more. It seems unlikely that differences in the type of tobacco smoked could account for the differences in mortality. The butts discarded in South Africa are, on average, longer than those discarded in Britain, but the difference in this respect is small.

Smoke pollution in the principal towns in South Africa is of the same order as that of many British towns and it is suggested that atmospheric pollution is the explanation for the large difference between the urban and the rural mortalities for lung cancer in South Africa. Heavy pollution in South Africa is of relatively recent date and the excess mortality among British immigrants under the age of 65 years may be due to exposure to heavier pollution in Britain before they emigrated. *Richard Doll*

CARTWRIGHT, Ann, MARTIN, F. M. & THOMSON, J. G. **Distribution and Development of Smoking Habits.** *Lancet.* 1959, Oct. 31, 725–7.

This paper is based on the results obtained from interviews of 125 persons aged 21 and 590 adults in Edinburgh. 3 in 4 men and 3 in 8 women smoked regularly. Women tended to become regular smokers at a later age than men. The largest male group of non-smokers was among men aged 21. The oldest male group had the largest proportion of smokers but their average consumption of tobacco was low, probably owing to expense. The lowest social class had the highest ratio of smokers to non-smokers and a greater proportion of heavy smokers who started smoking at an earlier age than in the other classes. There were no differences in the smoking habits of single and married women or between those in paid employment and those engaged solely in household duties. Smoking was given up for financial reasons rather than on health grounds, although 2/5 of all smokers said that they would like to give up smoking if they could do so easily.

[The statements in this paper depend on relative values and no absolute figures are given. This makes it impossible to assess the significance of some of the statements since some of the percentages must have been based on small numbers.] *W. J. Martin*

SANDERUD, K. **Squamous Metaplasia of the Respiratory Tract Epithelium. An Autopsy Study of 214 Cases. 2. Relation to Tobacco Smoking, Occupation and Residence.** *Acta Path. et Microb. Scandinavica.* 1958, v. 43, No. 1, 47–61.

The occurrence and extent of squamous metaplasia of the bronchial mucosa was examined at autopsy in relation to the history of smoking, occupation and place of residence. The incidence of metaplasia was found to be higher in smokers than in non-smokers (80% against 54% in men, and 2 out of 4 against 26 out of 63 in women).

Extensive—grade III—metaplasia was greater in cigarette smokers (53%) than in pipe smokers (30%) and "non-smokers" (18%) and was much greater in men who had smoked 25 or more cigarettes a day (100%) than in men who had smoked 15–24 cigarettes a day (50%) or 5–14 cigarettes a day (43%).

Metaplasia occurred more often among men engaged in dusty work than among professional and clerical workers or among open air workers. Metaplasia also occurred more often in men resident in Bergen than in men who had lived outside. It was, however, difficult to separate the effect of residence from that of occupation and the effect of occupation from that of smoking.

[It is difficult to assess the exact meaning of these results in the absence of data relating the incidence of metaplasia to age; it is, however, clear that differences in age distribution cannot wholly account for the different results for the various categories of smokers.]

Richard Doll

MALLET, L. & HÉROS-DEKEIREL. Présence d'une substance ayant les caractéristiques spectrales et chromatographiques du benzo-3,4-pyrène dans les extraits des tissus humains (poumons, ganglions, foie, rate). [**Presence of a Substance having the Spectroscopic and Chromatographic Characteristics of 3:4-benzpyrene in Human Tissues**] *Bull. Acad. Nat. Méd.* 1959, v. 143, Nos. 13/14, 295–300, 5 figs.

Previous studies have shown that 3:4-benzpyrene is present in (1) extracts of atmospheric smoke obtained from a large industrial centre, (2) the products of combustion of cigarette papers and of cigarettes, and (3) the fumes from internal combustion engines.

Extracts from tissues taken from Paris residents at autopsy have been examined by fluorescent spectrophotometry after separation by adsorption chromatography. The results showed amounts of 3:4 benzpyrene per gm. (dry weight) to the extent of:

(1) 0·1 μgm. in pulmonary tissue,

(2) 1·0 μgm. in tissue from the bronchial and medias-tinol glands. Greater quantities were found in old persons than in young, but the substance was detected in glands from subjects aged 14, 18 and 28 years,

(3) 0·05 μgm. in the liver of a boy aged 18 years and 0·015 μgm. in the liver of a girl aged 14 years, and

(4) 0·014 μgm. in splenic tissue from a man aged 60 years.

Practically no benzpyrene was detected in malignant hepatic deposits.

It is concluded that a substance of external origin having the characteristics of 3:4-benzpyrene can be found widely throughout the body and that the mediastinal glands provide the principal locus for the storage of the substance derived from inhaled material.

Richard Doll

JOHNSTONE, R. A. W. & PLIMMER, J. R. **The Chemical Constituents of Tobacco and Tobacco Smoke.** *Chem. Reviews.* 1959, Oct., v. 59, No. 5, 885–936. [419 refs.]

See also p. 237, ECKARDT, **Industrial Carcinogens.**

PHILLIPS, T. A. **Leukaemia and Geography.** *Lancet.* 1959, Oct. 24, 659–61, 4 figs. [11 refs.]

Between 1950–53 and 1954–57 the leukaemia mortality in England and Wales increased by 13%. The rate of increase varied in different areas; among the 52 counties (with their enclosed County Boroughs) there were 5 in which the crude death rates increased by over 50%—*i.e.* Westmorland (140%), Caernarvonshire (80%), Montgomeryshire (65%), Monmouthshire (65%) and Cumberland (59%)—and 3 others in which the increase was over 30%, *i.e.* Cambridgeshire (39%), Breconshire (38%) and Durham (38%). In nearly all the counties the initial levels were low, and the rate rose to a level substantially above the national average in only 1. It may, however, also be noted that of the 5 counties which showed the greatest increases 4 are mountainous and have a high annual rainfall, and much of the fifth county (Monmouthshire) is similar in character. It has been found that mountain areas with high rainfall show high levels of radioactive strontium (^{90}Sr) in soil and vegetables and that milk from such areas may contain 3 to 4 times as much ^{90}Sr per gm. of calcium as the national average. It is known that ionizing radiations can cause leukaemia and it is suggested that differences in radiostrontium fallout may be a cause of the variation in the rate of increase in leukaemia mortality. If this is so it must be presumed that small doses of radiation have a disproportionately great effect in comparison with large doses, since the available evidence on the effect of large doses would suggest that the expected increase in leukaemia mortality would be not more than 1/600th of the increase actually observed.

[In so far as the differences in mortality between areas are due to random factors it must be expected that those areas in which low mortalities are recorded in one period will tend to show the greatest increases. The author points out that the initial rates in the areas with large increases were, in fact, nearly all low and the number of deaths recorded in these counties were mostly small. It is, therefore, not unreasonable to suppose that the second observations are largely due to chance fluctuations. Another factor may be the extension of the pathological services which has followed the introduction of the National Health Service. It is possible that the diagnosis of leukaemia was initially less complete in scarcely populated and mountainous districts.]

Richard Doll

MISCELLANEOUS DISEASES

SAUER, W. & RHINOW, Elsa. Beitrag zur Frage des Kropfvorkommens durch Urochrome. [**Urochrome as a possible Cause of Endemic Goitre**] *Städtehyg.* 1959, Nov., v. 10, No. 11, 219–22, 6 figs. [10 refs.]

It has been stated in the literature particularly by HETTCHE [this *Bulletin*, 1956, v. 31, 310, 717] that endemic goitre may be caused by the presence in drinking water of urochrome derived from faecal pollution.

To test this theory a large number of drinking waters were bacteriologically and chemically examined in the North Rhine district. The results obtained by Rhinow have been published. Urochrome was found in 148

drinking waters from sources which are here described with some photographs. A survey was made to determine the incidence of endemic goitre among the people using drinking water from 29 sources with a high urochrome content and to compare this with the incidence in persons using water from 7 sources which had a low urochrome content. A table sets out the results of the survey. Among 83 persons using water of high urochrome content there were 65 (78·4%) who had goitre of varying degree as compared with only 22 (21·1%) persons with early goitre among 104 using water of low urochrome concentration. The findings are discussed with clear emphasis on the need for much more detailed investigation to test the validity of the theory of causation of endemic goitre by ingestion of urochrome. There are many and complex aspects of this causation which should be studied. *M. E. Delafield*

MENTAL HEALTH

PARGITER, R. A. & HODGSON, T. D. **The Mental Welfare Officer and the Psychiatrist.** *Lancet.* 1959, Oct. 31, 727–8.

"The Younghusband Committee thought that, until formal courses of training for mental health officers can be arranged, they should be encouraged to take part in outpatient psychiatric clinics." This paper is a short account of experience of a scheme of this kind in Huntingdonshire where in January, 1957 the county council appointed 2 officers to the combined posts of district welfare officer and mental welfare officer. In each week 1 of these officers attends the weekly outpatient clinic and sits with the psychiatrist while he is interviewing patients. Despite initial misgivings that the presence of the officer would prevent a full and frank history from being obtained experience has shown that this was not so except in rare instances. Any disadvantages are far outweighed by the advantages. The advantages are described shortly but clearly. The officer who has a dual purpose sees the patient from the beginning and thus his "presence at the first interview paves the way for his acceptance as a friend and counsellor by patient and relatives. . . . He acts as an essential link between the psychiatrist and all the local authority services and departments, including the police." Family doctors of the area also appreciate that the medical welfare officer is a valuable help in many ways.

"We have found the work rewarding and we believe that it has increased the efficiency of the service and its value to our patients." *M. E. Delafield*

Sanitation

The order in this section is:—**General; Water and Swimming Baths; Sewage, Trade Waste and Refuse Disposal; Housing; Atmospheric Pollution; Port, Ship and Aviation Hygiene; Infestation.**

GENERAL

WILSON, T. S. & CARTER, H. S. **A Survey of Hairdressers' Establishments in Central Glasgow.** *Med. Officer.* 1959, No. 13, v. 102, No. 20, 249–53.

A general survey of 50 hairdressing establishments (20 catering for men only, 20 for women only and 10 for both sexes) was carried out in the Central Division of Glasgow from May 1958 to March 1959.

There is a description of the methods employed in the survey and of the bacteriological examination of different items used in the establishments. Usually swabs taken from 7 items such as brushes, razors, combs, scissors and curlers were examined in each case. The findings of the survey are reported in considerable detail. In the discussion it is stated that many of the hygienic methods adopted in the shops visited were inadequate and although almost all shops made some attempt at disinfection the bacteriological findings showed that it was not very successful. It is suggested that a solution of quaternary ammonium compound, which appears not to be used in any of the shops, would give the best results.

Staff accommodation and toilet arrangements for customers and staff were often found to be inadequate by modern standards. In the bacteriological survey of 414 implements used in the establishments it was found that those for women were cleaner, as judged by the total bacterial count than those catering for men. *Staphylococcus aureus* was isolated frequently, more often from men's than from women's saloons.

Suggestions are made for improving the hygiene of hairdressing establishments in Glasgow where no bye-laws relating to these exist at present.

M. E. Delafield

WATER AND SWIMMING BATHS

(Including the Bacteriology of Water Supplies)

GRAY, R. D. **Formate Lactose Glutamate: a Chemically Defined Medium as a Possible Substitute for MacConkey Broth in the Presumptive Coliform Examination of Water.** *J. Hygiene.* 1959, Sept., v. 57, No. 3, 249–65. [21 refs.]

As a result of investigations of modifications of the Folpmers glutamic acid medium by the Public Health Laboratory Service Water Sub-committee [this *Bulletin*, 1959, v. 34, 67] lactose glutamic acid medium was shown to yield results more slowly than MacConkey broth and with fewer total presumptive positives. A recommendation was made to seek further modifications to make this medium less inhibitory to true coliform organisms. This has been achieved in experiments described in this paper.

The Folpmers glucose glutamic medium was at *p*H 6·0 and all modifications described before this paper have also been at *p*H 6·0. With lactose instead of glucose it was thought that this low *p*H might be too inhibitory and at *p*H 7·5 it was shown that gas was produced earlier and in greater volume. Later experiments showed the optimum to be *p*H 6·7. Several additives were tried in an attempt to improve performance but the only one of any value was sodium formate. Gas production was earlier and more abundant. The optimum concentration was 0·025% sodium formate. Lower concentrations gave less gas and higher concentrations retarded acid formation owing to the buffering action of the formate.

In the first series of experiments MacConkey broth was compared with formate lactose glutamate at *p*H 7·5; lactose glutamate at *p*H 7·5 and glucose glutamate at *p*H 6·0. Special care was taken with sterilization to avoid hydrolysis of lactose. Comparisons were made on single 10 ml. tubes of each medium and positive results were obtained in 361 samples. The three glutamic acid media produced more *Escherichia coli* than MacConkey broth in 48 hours and the formate and glucose media produced at least the same number of true coliform organisms with fewer false positives. The lactose glutamate, as in previous trials, produced fewer true coliforms. MacConkey broth gave the largest yield in 18 and 24 hours but at 24 hours the formate medium was not far behind MacConkey broth and appreciably ahead of the glucose medium. In the second series formate lactose glutamate at *p*H 6·7 was compared with MacConkey broth in which 134 samples gave positive results. The formate medium again yielded an increased total of *E. coli* and true coliforms; also the results with this medium at 18 hours were of the same order and at 24 hours ahead of those obtained from MacConkey broth. With certain reservations, the modified medium is recommended for replacement of MacConkey broth in the presumptive coliform test of water.

E. Windle Taylor

SCOTT, W. J. **Connecticut studies its Shore Bathing Waters.** *Connecticut Health Bull.* 1959, Aug., v. 73, No. 8, 219–29, 3 figs.

In 1957 and 1958 further studies were done along the Connecticut shore as in previous years [see this *Bulletin*, 1951, v. 26, 702; 1953, v. 28, 964; 1958, v. 33, 252]. In 1957, bacteriological samples of sea water were collected from 600 sampling stations and in 1958 from 505 stations. Samples were collected from a rowing boat in water 2–6 feet deep, refrigerated and taken to a laboratory for examination. Mainly on the basis of sanitary survey, the Connecticut coast was divided into sections varying in length from 2,000 ft. to one or more miles. These sections covered 2–48 sampling stations.

The classification of these sections was done by the same criteria as in previous surveys and the results obtained are summarized as follows.

Class	Most Probable No. Coliforms per 100 ml.	Mileage	Percentage
A	0–50	62·0	24·5
B	51–500	143·2	56·6
C	501–1,000	25·3	10·0
D	over 1,000	22·5	8·9

The author goes on to describe the present situation as regards coastal sewerage systems. All the sewage from municipal sewerage systems along the Connecticut coast is subjected to treatment, including at least sedimentation and chlorination, except at one or two points of overflow on sewer collecting systems where improvements are planned, and in a few communities with many private sewers but no public sewerage. *B. Moore*

CLARKE, N. A. & LANG, S. L. **Enteric Viruses in Water.** *J. Amer. Water Works Ass.* 1959, Oct., v. 51, No. 10, 1299–317. [88 refs.]

The purpose of the authors of this long paper with its numerous references was "to review and record the present status of knowledge regarding the relationship of water to human virus diseases". The 2 main points of consideration of this subject are: "(1) the epidemiological implications of water supplies in the transmission of virus diseases, and (2) the adequacy of current water treatment methods in removing or destroying viruses in water".

The term "enteric virus" as used in this paper includes all human viruses which are found in quantity in human faeces and in sewage. The characteristics of these enteric viruses are described and it is stated that proved water-borne outbreaks of enteric-virus disease have only occurred with infectious hepatitis virus although suspected water-borne outbreaks of poliomyelitis have been reported [this *Bulletin*, 1954, v. 29, 799]. Separate sections of the review deal also with

the adenoviruses, Coxsackie, ECHO and other viruses. Some of these may be considered as potential water-borne viruses.

There is next a description with some tables of data of the extent to which certain viruses may be destroyed in different stages or processes of water purification *i.e.* storage, flocculation, activated-carbon, filtration, chlorination, chlor-ammonization, chlorine dioxide addition, elemental iodization, ozonization and heat treatment. As is stated in the summary the information obtained in experimental studies is as yet extremely limited.

M. E. Delafield

SEWAGE, TRADE WASTE AND REFUSE DISPOSAL

J. Amer. Water Works Ass. 1959, Oct., v. 51, No. 10, 1251–4. **Effects of Synthetic Detergents on Water Supplies. Task Group Report presented on July 14, 1959, at Annual Conference, San Francisco** [Haney, P. D., Chairman].

A Task Group of the American Water Works Association, which will attempt to keep in touch with future developments, here reports on some present aspects of the effects of synthetic detergents (syndets) on water supplies. Sales of these detergents in the United States now represent 72% of the total market for cleansing agents and in 1959 it is estimated that about 20 lb. per person will have been used. Some account is given of researches which have been carried out on the pollution of ground water by syndets and many other chemicals.

An important aspect of the detergent problem is the resistance of surface active agents, of which alkyl benzene sulphonate (ABS) is the most widely used, to biochemical degradation. This problem is being studied and manufacturers also are making a serious attempt to develop alternative " surfactants " which are as efficient as ABS and also susceptible to biochemical degradation in sewage treatment. Special mention is made of the findings of a British technical committee (Second Progress Report of the Standing Technical Committee on Synthetic Detergents H.M.S.O., London, 1959) in the development of a new ABS, which apparently differs only " with respect to chemical structure and is entirely satisfactory for use in detergent manufacture ". Tests showed that the new ABS had much less tendency to cause effluent foaming and that trickling filters removed 94% of it as compared with only about 67% of conventional ABS.

M. E. Delafield

Bringmann, G. & Kühn, Renate. Die Überlebensrate von Coli-Bakterien auf Freiland-Pflanzen in Abhängigkeit von Zeit und Witterung. [**The Survival of Coliform Bacteria on Plants in Relation to Time and Weather**] *Gesundheits-Ingenieur.* 1958, May 10, v. 79, No. 5, 148–50, 4 figs.

The experiments described in this paper were carried out on white clover plants to determine the survival time of coliform organisms on the leaves of the clover after spraying with mechanically clarified sewage. As the determination of the number of bacteria on the leaves is not practicable, the method adopted to assess the degree of infection was to examine large numbers of leaves for the presence of coliform organisms. The results of the experiments are described with figures. It was found by the examination of 2,500 single leaves before watering with sewage that about 8% of these were normally infected by natural circumstances. In a period after watering with sewage the examination of 20,000 leaves showed that the percentage of infected leaves decreased fairly rapidly and reached the " normal " of about 8% in about a fortnight. Other observations indicated that in periods of high relative humidity the proportion of infected leaves was increased but was diminished in periods of sunshine.

M. E. Delafield

Bringmann, G. & Trolldenier, G. Quantitative Coli-Befunde an Futterpflanzen in Abhängigkeit von Mehrfachberegnung, Bestandsdichte, Bodeninfektion und Luftfeuchte. [**Experiments on the Survival of Coliform Organisms on Forage Plants Watered with Sewage**] *Gesundheits-Ingenieur.* 1959, Oct. 10, v. 80, No. 10, 306–9, 5 figs.

In previous work it was shown that an important factor in the survival of coliform organisms on forage plants sprayed with sewage was the relative humidity of the atmosphere. The experiments which are fully described in the present paper had as their object the study of the influence of other factors on the survival of bacteria on the leaves of white clover plants watered with clarified sewage. Some of the factors examined were (1) the closeness of the plants to each other, (2) the frequency of watering and (3) secondary infection of the plants from the soil in which they were grown. There is a full discussion of the results of this complex study in which over 52,000 clover leaves were examined bacteriologically. In general it was found that the number of coliform organisms on the leaves after watering lessened during the following 14 days when the infection rate of the leaves reached a constant value which persisted for the succeeding 4 weeks of the study. The modifications of this general finding brought about by the factors which were the subject of study are fully considered. *M. E. Delafield*

Ministry of Housing and Local Government. **The Control of Radioactive Wastes.** With an appendix by Radioactive Substances Advisory Committee, Panel on **Disposal of Radioactive Wastes** [Key, A., Chairman]. Cmnd. 884. 46 pp. 1959. London: H.M. Stationery Office. [2s. 6d.]

This is the report of a Panel set up by the General Purposes Sub-Committee of the Radioactive Substances

Advisory Committee in 1956 with these terms of reference: "To ascertain the nature and quantity of radioactive waste likely to arise in the foreseeable future; to advise on the best methods of securing that the waste is disposed of safely; and to advise whether any new legal provisions or amendments to existing legislation are necessary to ensure safe disposal and, if so, to advise on the form which the new provisions or amendments should take". The report was presented to Parliament and the "Government have accepted the panel's recommendations in principle, and now seek to give effect to them in the Radioactive Substances Bill".

The report has sections entitled (1) introduction, (2) existing legislation on waste disposal, (3) the production of radioactive wastes, (4) best methods of disposal, and (5) suggested method and powers of control. These sections have many sub-sections, the subject matter of which is given clear, expert consideration which is necessarily made in scientific and technical terms.

The most important general principles emerging from the report are summarized in the foreword and among these are: "It is not in the nature of radioactivity that the economic value of the useful materials involved can be realised without some release of ionising radiations to the general environment. But this release can be controlled within safe limits." and "The indications are, therefore, that control ought to be organised on a national basis as a Government responsibility".

It is reassuring to read: "It is worth noting that despite the rapid development of nuclear power in this country and elsewhere, there is not a single case on record in which the use of nuclear fission for peaceful purposes has caused hurt from radioactivity to any member of the public at large". *M. E. Delafield*

HOUSING

MINISTRY OF HOUSING AND LOCAL GOVERNMENT. **Caravans as Homes.** A report by Sir Arton WILSON, K.B.E., C.B. July 1959. Cmnd. 872. pp. v + 83. London: H.M. Stationery Office. [5s.]

This is the report on an investigation commissioned in November 1958, which is "about people (other than gypsies or vagrants) who live in caravans and have no other homes. It does not deal, except in passing, with caravans used merely for holidays or weekends. It is only a factual survey of the situation and its problems and the views that people hold about them without recommendations. And it relates only to England and Wales". Information for this investigation was not obtained formally or in public but from a wide range of national organizations, by questionnaires to local authorities, by visits to over 200 caravan sites and interviews with many people concerned with caravans.

The report contains a wealth of information which is presented clearly and in interesting fashion. Its 10 sections with many sub-sections have the titles (1) *Introductory*, (2) *The Extent to which Caravans are used for any Purpose*, (3) *The Extent to which Caravans are used as Homes*, (4) *The People who make their Homes in Caravans*, (5) *The Caravan Homes*, (6) *The Residential Caravan Sites*, (7) *Problems of Control*, (8) *Present Public Policy towards Caravan living*, (9) *Views of the Interests Mainly Concerned* and (10) *Summing up*. There are 2 maps which show the distribution in 1959 of sites containing 3 or more residential caravans and the distribution of major postwar increases of population and industry. Some of the facts given in the summary are that at present about 150,000 people comprising 60,000 households in England and Wales, not gypsies or vagrants, are living in caravans on about 13,000 plots of land mainly in rural or semi-rural areas close to the more rapidly expanding industrial centres—particularly Greater London. The increase in caravan living shows no sign of slackening. Caravan dwellers consist mainly of young or youngish married couples often with 1 or 2 infants and rather less often with older children. The majority hope to move into houses, flats or bungalows after a few years at most. More than half the caravan dwellers live in modern caravans which are of improved quality and equipment. A small minority of the sites are really well located, attractive, and well-organized; a small minority are unpleasant, insanitary and ill-run and the rest are unsatisfactory in some way.

The average price of a caravan on hire purchase is £500 but the range of price for a fully or partly furnished van is £360 to £2,000. Weekly site rents charged by site operators range from 7s. 6d. to 30s.

Among the problems which are stated are the unsuitability of a number of caravans and caravan sites, inadequacy of the regulations of control and the difficulties inherent in the fact that local authorities in general disapprove of caravan living in principle.

Local authorities are, however, "tending increasingly to accept the view that for some years ahead there will continue to be a large number of people still living in caravans" but "believe it to be essential, in any event, that their powers of control and enforcement both in the public health and the planning field should be strengthened very considerably". *M. E. Delafield*

GISBORNE, A. C. F. **Caravan Homes 1959.** *Sanitarian.* London. 1959, Nov., v. 68, No. 2, 94–110, 16 figs. [11 refs.] Discussion 110–16.

This paper was presented at the Conference of the Association of Public Health Inspectors in September 1959. It is restricted to consideration of caravans used by people who have no other home including gypsy-dealers but excluding Romanies and showmen. The author from his experience as Senior Public Health Inspector and Housing Officer, Bushey Urban District Council, examined the problems of adequate control, health, and welfare in caravan homes which have increased so greatly in number in the last decade. The types of residential caravan are described with suggestions about new vans in respect of their construction,

size, hygienic provisions and general amenities. Sections of the paper were devoted to the private site, the municipal site and the site of the future—their advantages, disadvantages and control. The gypsy-dealer problem was also discussed. Under the heading of legislative control of all types of caravan and of sites it was stated that "there is an overwhelming case for the revision of legislation, both from the planning angle and the public health side". To illustrate the points made in the paper there are a number of photographs of good and bad caravans and sites, some plans of caravan sites and appendices of relevant recommendations, notes and conditions issued by the Hertfordshire County Council and the Bushey Urban District Council.

There followed a long and vigorous discussion.

M. E. Delafield

INFESTATION

BENTLEY, E. W., BATHARD, A. H. & RILEY, J. D. **The Rates of Recovery of Sewer Rat Populations after Poisoning** *J. Hygiene.* 1959, Sept., v. 57, No. 3, 291–8, 1 fig.

This paper is from the Infestation Control Division, Ministry of Agriculture, Fisheries and Food. In it there is a description of observations relating to 8 sewer rat populations "and, in particular, to three of them that were allowed to develop undisturbed for nearly 2 years after knock-down". This work was undertaken in order to obtain more knowledge of the rate of recovery of *Rattus norvegicus* populations in sewers after reduction by poisoning since this knowledge would facilitate the evaluation of alternative devices which have been suggested for the more economical control of these rats. The method of investigation was the same as in previous studies (*J. Hygiene*, 1953, v. 51, 483, *ibid.*, 1958 v. 56, 19; *Ann. Applied Biol.*, 1955, v. 43, 485), to carry out a "census" before and at different periods of time after the poison treatment by recording for several days the 24-hour consumption of wheat from a surplus maintained at the bottom of about 50 widely scattered manholes. The results of this study which are clearly described and discussed led to the conclusions: (1) "In three of the systems the mean rate of increase was of the order of 3% per week. This is attributed solely to breeding and is regarded as typical for rats in sewers" and (2) "Higher rates of increase (up to 11·8%) were also recorded but are believed to have been partly due to immigration or to have been only apparent (as a result of census difficulties)."

M. E. Delafield

Occupational Hygiene

The order in this section is:—**General; Occupational Physiology; Occupational Diseases (Dust Diseases, Poisoning, Skin Diseases, Others); Occupational Psychology; Heating, Lighting and Ventilation.**

GENERAL

DRINKER, P. **Industrial Hygiene and the Practice of Occupational Medicine.** *Arch. Indust. Health.* Chicago. 1959, Oct., v. 20, No. 4, 271–8.

In the George Gehrmann Lecture presented to the American Academy of Occupational Medicine, the speaker reviewed his 40 years' experience in industrial hygiene which he called a child of preventive medicine. The founders were physicians who worked for safe and healthy working conditions. At one time industrial hygiene was limited to the determination of atmospheric contamination. Industry has, however, integrated preventive measures into production plans, and the production manager often needs a man who can devise, install and operate control equipment. This has tended unfortunately to separate these engineering specialists from the medical department, though they should not be regarded as being unfamiliar with problems of occupational medicine.

The development of occupational medicine since the industrial revolution of Britain shows how reform overcame early evils of mechanization, such as overcrowding in the mill-towns, sanitation problems, child labour and industrial diseases. Somewhat similar new-town problems had to be overcome during wartime. Nowadays in less well developed countries it may be as necessary for a new industrial enterprise to arrange homes, schools and hospitals for workers' families as to set up occupational health services for the workers themselves. Industrial morale, which is the concern also of the industrial medical officer, may depend on this.

The American standard of living and quick pace of work mean that poor working conditions are productively and economically unsound: modern mechanization makes possible safe environmental control with the consequent disappearance of costly compensatable illnesses. Reminiscences give glimpses of how good

industrial hygiene standards were achieved and of their results. The changing attitude of labour organizations to health control leads to the hope that it may be possible to insure, in all senses of that word, against illness; the worker has become interested in preventive medicine and in keeping well. [This paper contains brief passing allusions to several hazards and their control.] *M. Patricia Fitzsimons*

AANONSEN, A. **Medical Problems of Shift-Work.** *Indust. Med. & Surgery.* Chicago, 1959, Sept., v. 28, No. 9, 422–7.

The medical problems of shift-work were investigated in 2 electro-metallurgical factories and 1 electro-chemical factory situated in a small industrial community in the western part of Norway during the period 1948–54. The 3 factories employ about 1,750 persons, of whom 1,106 are included in this study. All employers and foremen were excluded from the investigation and so were the labourers who began working at the factories after 1948 and those less than 20 years old. The 1,106 persons in question are classified as follows:

	Number of men.
Day-labourers	345
Shift-labourers	380
Day-labourers (previous shift-labourers) ...	350
Alternating day- and shift-labourers ...	31
Total	1,106.

The 350 day-labourers (previously shift-labourers) are classified into two groups, A and B. Group A consists of 128 labourers, having been dispensed from shift-work owing to ailments incurred during this kind of work. Group B comprises 222 labourers who " more incidently " have passed from shift to day-work. The author points out that the 3 main groups in the above table have approximately the same number of persons, and that the age distribution allows a comparison, but he gives no figures in support of the latter statement.

The study includes routine annual medical examinations and investigation of all the sickness absence records during the year 1952. In addition, all men who at the beginning of 1952 were between 50 and 65 years of age have had their sickness records for the years 1939–51 examined—the help of the local health insurance company was obtained in this respect. This retrospective study did not include short-term absence (absences of 1, 2 or 3 days' duration). The author also collected information on housing conditions and sleeping habits.

There was no increased morbidity with regard to disorders of the nervous or alimentary systems or with regard to the heart among regular shift-labourers. This group also showed a lower frequency of absence from work (30%) and medical consultations, as compared with the day-labourers. This is attributed to the fact that the 380 regular shift-labourers represent selected material, because 350 previous regular shift-labourers had been transferred to day-work. Of these, 128 were removed for medical reasons. Within this group there was a marked increase in the frequency of nervous and alimentary diseases. There was, however, no increase in heart diseases in this group as compared with those day-labourers who had never been subjected to regular shift-work. The study clearly shows that the reason some people were not able to go on with shift-work was lack of sleep. *P. J. R. Challen*

RAMSAY, H. T. **International Mines Safety Research. 11th " W. M. Thornton Lecture " delivered before Ass. Mining Electrical & Mechanical Engineers at Newcastle upon Tyne on 12th June 1959.** 17 pp., 23 figs. [60 refs.] 1959. Ministry of Power, Safety in Mines Research Establishment.

Research on safety in mines assumed an international character at a very early stage when scientists and mining engineers corresponded with others interested in particular aspects of safety in mines. Collaboration became more formal in 1908 when the Technologic Branch of the U.S. Geological Survey invited representatives of Belgium, Germany and Great Britain to advise on the work to be carried out at the new research station at Pittsburgh. In 1912 the U.S. Bureau of Mines called a conference of countries that had experimental stations, and Austria, Belgium, France and Germany accepted. In 1923 a formal agreement was reached for co-operation between Great Britain and the United States. In 1931 a further conference was held at Buxton, and that was attended by representatives of Belgium, France, Germany, the United States and Great Britain. At this conference a general interchange of reports and information was arranged, and this was to be supplemented by the interchange of visits and where appropriate by the interchange of personnel. It was also agreed that meetings would be held in each country in turn. The last conference was attended by representatives of Austria, Belgium, Canada, East Germany, Finland, France, Great Britain, Holland, India, Italy, Japan, New Zealand, Poland, Saar, South Africa, United States, West Germany, and Yugoslavia, and by a representative of the International Labour Office.

The organization, location, and main programmes of the various national research laboratories are outlined. International research on safety in mines is illustrated by reviews of four fields which are primarily those in which disasters may arise. These are coal-dust explosions, mining explosives, fire-damp ignitions, and fires.

The directors of research and their senior colleagues have, except during the war years, kept in close personal touch. Informal reports are exchanged freely and visits are made whenever the need arises. An example of the value to Britain of this friendly collaboration is the way in which both Germany and Poland freely provide the results of their experimental-mine researches on coal-dust explosions and stone-dust barriers. These results supplement the British laboratory studies and make a great difference to the confidence with which they can be regarded. Improved methods of testing explosives are widely discussed, and each country profits by the knowledge and experience of the others. This

collaboration has speeded the research and advanced the cause of safety in all the countries involved.

Thomas Bedford

CROTON, L. M. **Artificial Respiration. A Guide to Current Practice.** *J. Inst. Sci. Technol.* London. 1959, Oct.-Dec., v. 5, No 4, 16–24, 11 figs. [25 refs.]

This article describes briefly the methods of artificial respiration which are known as the Holger Nielson, Schafer, Silvester and mouth-to-mouth insufflation. Interest in this subject at the moment centres on the powerful claims being made that the mouth-to-mouth method produces a far bigger tidal volume of air in the lung than is obtained by any push and pull method. In consequence there is a very much more rapid transference of O_2 to the blood of the asphyxiated subject.

The author, in a written communication, tells of an opportunity he has had to convince himself of the validity of this claim by giving artificial respiration to an anaesthetized and curarized patient before a surgical operation.

Training of first-aid workers in the mouth-to-mouth method presents some difficulties on social grounds but it is hardly justifiable to call them "obvious ethical disadvantages"—no doubt the author meant aesthetic. In any case these difficulties are being surmounted and many in this country are already trained in the mouth-to-mouth method.

T. O. Garland

FOX, F. W. **Changing Concepts in Nutrition and Dietetics: some possible Applications to the Feeding of Native Mine Labourers.** *Proc. Mine Med. Officers' Ass.* Johannesburg. 1959, May–June, v. 39, No. 369, 1–18, 2 charts. [30 refs.] Discussion 18–20.

This is the text of an address given to medical officers of the mines by the doyen of South African nutritionists. The first part ranges widely over the changes in our attitude towards the science of nutrition in the last 50 years. It is suggested that the standards of requirements of nutrients laid down in text-books are often too high and could be reduced. High intakes, far beyond minimal needs, are not necessarily advantageous.

The second part of the lecture gives a brief résumé of trends in the policy of feeding miners on the compound. It is emphasized that there is need for research on the relationship between diet and physical efficiency. Enough attention may not have been paid to providing a variety of dishes and to meeting the miners' accustomed likes and prejudices.

R. Passmore

OCCUPATIONAL PHYSIOLOGY

MISSENARD, A. **On Thermally Equivalent Environments.** *J. Inst. Heating & Ventilating Engineers.* 1959, Nov., v. 27, 231–7, 6 figs. [19 refs.]

Professor Missenard's work may well prove to be the basis of a set of standards, more complete than those already available, by which thermal comfort and thermal stresses may be evaluated. It is valuable for heating and ventilating engineers, as for practitioners in occupational hygiene, particularly those connected with hot industries, to be given criteria for their designs obtained from physiological experiment.

For more than 20 years, as the author points out, the air conditioning industry has depended upon the American nomographs relating to "effective temperature" (a physiological parameter), but the experiments by which this standard was determined were made by subjects who passed to and fro between 2 air conditioning rooms—a null point being decided when the subjects agreed that they experienced the same sensation of warmth, or of cold, in each room. The author points out that in these classical experiments YAGLOU and his co-workers omitted the important component of radiant heat gains or losses from or to solid surrounds. [But BEDFORD (see this *Bulletin*, 1936, v. 11, 735) has shown that a practical, if empirical, remedy for this deficiency may be found by using the readings of a globe thermometer in place of those of a glass, or silvered, thermometer on the effective temperature charts; a value then being obtained which Bedford calls the *corrected effective temperature*.]

Missenard, however, raises the more serious objection that the thermal effects of environments are more likely to be verified after a considerable period of exposure rather than in transient passage. The effects may not be comparable. (It may be relevant to recall that it has been shown that when clothing materials are placed over a warm, dry surface more than two hours may elapse before complete thermal equilibrium is established [see ANGUS, this *Bulletin*, 1937, v. 12, 233].) DUPUY (Communication au 6ème Congrés de Chauffage et Ventilation, Paris, 1937) is quoted as having proved that what the author terms *equivalents de passage* show no agreement with the sensations of thermal equivalence (*equivalents en séjour*) of acclimatized subjects. WINSLOW, HARRINGTON and GAGGE [this *Bulletin*, 1938, v. 13, 21, 22] are quoted as having measured a parameter defined as "operative temperature" with acclimatized subjects; arriving at a similar conclusion. Winslow used the term "biothermal equivalent", and stated that this is related to the wetness of the skin (for naked subjects) and not to its temperature. The author stresses the importance of the fraction of the area of the body that is wetted with sweat.

American workers have shown that in a calm atmosphere the cutaneous evaporation depends only on the air temperature, whatever the humidity may be. It is also recognized that in such environments metabolism is independent of humidity; from all of which Missenard concludes that the wetted fraction will be greater the greater the humidity of the air. To give

a physical measurement of resultant temperature a "wet resultant thermometer" is described and illustrated, in which a black metal globe containing a thermometer carries a number of wetted wicks, by altering the number of which a larger or smaller area of the globe may be wetted. [The author gives no indication as to how much of the surface should be so wetted when making measurements.] Such an instrument is sensitive to radiant heat, as well as to the effects of humidity.

2 nomographs, similar in construction to the well known nomographs used for finding effective temperatures, are given—the protocols needed being dry bulb temperature, wet bulb temperature and air velocity. One of these charts relates to acclimatized, naked, sedentary subjects; the other to acclimatized, clothed and moderately active subjects. For both charts it is assumed that air and walls are at the same temperature. It may be noted that although the author stresses the importance of the radiation components no provision for such has been made on these nomographs. It is not even suggested that globe thermometer temperature may be substituted for the dry bulb air temperature.

Missenard's work was restarted, after the war, in 1946, and was carried out in psychrometric chambers in which 6 subjects stayed for considerable periods. It is stated that the human body can judge the equality of 2 sensations separated by the half hour or so needed for adaptation.

The high air velocities shown in the nomographs are, in the reviewer's opinion, open to criticism. The line of lowest air speed is for "calm" air, but it is noted that this is defined as air moving at less than 39 feet per minute. The other 4 velocity lines are for 98, 197, 393 and 591 feet per minute. It has been found that even in hot factories people will seldom tolerate air velocities of over 100 feet per minute. ANGUS and BROWN [this *Bulletin*, 1958, v. 33, 147] have shown that in an occupied lecture room an increase of air velocity from about 10 to about 20 feet per minute gave to seated students very real increased subjective sensations of coolness and freshness, and, perhaps, a slight sensation of decreased humidity. In most occupied enclosures air velocities above 50 feet per minute are rare, and it would seem that in future work on this important subject the range of air velocity between 10 (or less) and 50 feet per minute should be given the closest attention as being of physiological, and, perhaps, psychological importance. One difference between the author's nomographs and those used for effective temperature is that in the new charts humidity has less effect on sensation at lower temperatures. Comparison with figures given by JENNINGS and GIVONI are made (*Amer. Soc. Heating & Air Conditioning Eng. J.*, January, 1959). *T. C. Angus*

MCGRATH, Margaret W. & THOMSON, M. L. **The Effect of Age, Body Size and Lung Volume Change on Alveolar-Capillary Permeability and Diffusing Capacity in Man.** *J. Physiology*. 1959, June 11, v. 146, No. 3, 572–82, 3 figs. [19 refs.]

The published figures for diffusing capacity for carbon monoxide in normal human lungs show a wide scatter, often for the same method of determination. Since the figure for diffusing capacity estimates the capacity of the alveolar-capillary membrane of the lung as a whole to transfer CO, differences in area of membrane between persons may account for a large proportion of the total variation. Few authors have made any attempt to allow for body size, but some have expressed the diffusing capacity per unit area of body surface area. In the present paper evidence is presented to show that the diffusing capacity depends on age as well as on body size, but that the permeability figure, "k", of KROGH (*J. Physiology*, 1915, v. 49, 271)—the time constant of the exponential of CO concentration during breath-holding—which also depends on age, appears to be independent of body size.

The permeability and diffusing capacity were measured in 39 normal male subjects of ages ranging from 15 to 75 years by the single-breath method of Krogh, with a portable box-bag apparatus earlier described by the second author (*ibid.*, 1958, v. 142, 17p). There was a highly significant fall in the alveolar-capillary permeability with age. A highly significant multiple regression was also obtained between age, surface area and diffusing capacity, but even with the allowance for surface area the prediction of diffusion capacity was less accurate than that of permeability from age alone. In 8 subjects the effect of altering alveolar volume over the widest range possible was studied. With lung volumes up to 1 litre from maximum the relation between permeability and alveolar volume appeared to be reciprocal, consistent with constant area, thickness and specific permeability of the membrane. Within 1 litre of maximum the permeability was relatively independent of the lung volume, as would occur if the membrane were stretching, but the diffusing capacity was critically dependent on the lung volume over this range.

The use of the permeability figure in preference to the diffusing capacity has the advantage that it makes unnecessary the measurement of lung volume.

Thomas Bedford

LEWIS, B. M., LIN, Tai-Hon, NOE, Frances E. & HAYFORD-WELSING, E. J., with the technical assistance of Erma FLAHERTY. **The Measurement of Pulmonary Diffusing Capacity for Carbon Monoxide by a Rebreathing Method.** *J. Clin. Investigation*. 1959, Nov., v. 38, No. 11, 2073–86, 9 figs. [32 refs.]

OCCUPATIONAL DISEASES

SCHMIDT, K. G. & HEIDERMANNS, G. Untersuchung von Staubproben mit dem Phasenkontrastmikroskop, insbesondere bei Verwendung von Membranfiltern. [**Evaluation of Dusts with the Phase Contrast Microscope, with Special Reference to the Use of the Membrane Filter**] *Staub.* Düssledorf. 1959, Dec. 1, v. 19, No. 12, 413–16, 2 figs.

The English summary appended to the paper is as follows:—

"The article describes a process for the phase contrast microscopic quantitative mineralogical determination of dust types on membrane filters. In this process the dusts are evaluated without modification of their aggregate condition. The necessary table of figures is given."

SCHMITT, K. Grundlegende Untersuchungen zum Thermalpräzipitator. [**Fundamental Studies on the Thermal Precipitator**] *Staub.* Düsseldorf. 1959, Dec. 1, v. 19, No. 12, 416–21, 10 figs. [15 refs.]

The English summary appended to the paper is as follows:—

"The article discusses the examination of particles of aerosols in a suspension condenser with temperature gradient. The movement of the particles in the temperature field can only be described where and when they are large or small against the free course length of the gas. The corresponding formulae are quoted. The results obtained are applied to the thermal-precipitator."

BATTIGELLI, M., BELLINI, F., FOSSATI, F. & GARAVAGLIA, C. Studio comparativo del "valore diagnostico" degli schermogrammi 7 × 7 cm e 10 × 10 cm e dei radiogrammi 35 × 35 cm nelle pneumoconiosi. [**A Study of the Diagnostic Value of 7 × 7 cm and 10 × 10 cm Photofluorographs and 35 × 35 cm Radiographs in Pneumoconioses**] *Med. d. Lavoro.* 1959, Aug.–Sept., v. 50, Nos. 8/9, 541–57, 2 figs. [23 refs.]

For the diagnosis of pneumoconiosis the comparative value of the 3 different sizes of X-ray photographs has been studied in a series which included 150 workers affected with pneumoconiosis, mostly silicosis, at various stages of development and some 10 other workers suffering from other affections of the pulmonary-bronchial system. The 2 smaller sizes are taken indirectly through a fluoroscopic screen.

Several X-ray pictures in each size were made from each patient, and the 3 to be used in the investigation were selected to show the same respiratory stage, the same degree of contrast and the least amount of blurring due to movement at the time of exposure. The paper gives details of the X-ray apparatus used which was the same in all cases.

A modification of the Cardiff-Douai system of classification has been followed. Based on the pattern of the X-ray shadow, they identified 12 categories, namely, a group of "simple" pneumoconiosis with 9 subdivisions (including one marked "normal") and another group of "massive" disease with 3 subdivisions.

Each of 6 radiologists, working independently, studied every X-ray picture, using a magnifying glass for the 2 smaller sizes. Each reader interpreted each picture twice on separate days and subjective variations were thus minimized.

The conclusions from statistical analysis, the results of which are summarized in tables, are briefly as follows: (a) In the diagnosis of massive types of pneumoconiosis there is no advantage of one miniature size of X-ray photography over the other. (b) In simple pneumoconiosis the larger of the miniature sizes has a slightly better diagnostic value. (c) In most cases the larger picture taken by direct methods is more helpful than miniature, indirect, photography in the diagnosis of pneumoconiosis. (d) Condensation of micro-nodules and an increased gradient of contrast may result in such optical effects as will make miniature, indirect, methods more valuable than the larger, direct, X-ray photography for diagnosis.

This investigation has shown the importance of the subjective factor in this kind of work. Previous experience of the correct diagnosis of pneumoconiosis from X-ray photographs gives an obvious proportionate advantage. The classification of the cases into 12 categories has proved quite adequate and, the authors of this paper consider, should be adopted internationally. *J. Cauchi*

FRANCIA, A., MONARCA, G. & CAVALLOT, A. Osservazioni clinico-roentgenologiche sull'associazione silicosi-sclerodermia. [**Clinical and Roentgenologic Observations on the Silicosis-Scleroderma Association**] *Med. d. Lavoro.* 1959, Aug.–Sept., v. 50, Nos. 8/9, 523–40, 11 figs. [Numerous refs.] English summary.

Various European authors have written about the association of rheumatism with silicosis, sometimes complicated by sclerodermic manifestations [this *Bulletin*, 1953, v. 28, 831; 1954, v. 29, 53]; in 1958, South African workers [*ibid.*, 1958, v. 33, 137] reported 16 cases of scleroderma in 8,000 gold miners, whereas 25,000 other patients seen at the Pretoria Hospital in the course of 4 years produced not a single case.

The authors of the present paper found 6 cases of scleroderma among 1,000 patients with silicosis. Scleroderma is liable by itself to impair the respiratory system and, indeed, cause lung fibrosis which it may be difficult at first to differentiate from silicosis; and, in its turn, scleroderma may escape diagnosis for a time after it has supervened in a case of pre-existing silicosis. Both diseases involve more especially the connective tissue system. These authors discuss the clinical, X-ray and pathological aspects of the two diseases and the reciprocal modification of the changes when both conditions are associated in the same patient, which they believe to be more common than is realized.

They give an account of each case, including the findings in one autopsy.

The onset of scleroderma in the course of pneumoconiosis accelerates the process of fibrosis and favours the supervention of tuberculosis. The authors endeavour to fit VIGLIANI's immunological theories of silicosis into the picture. *J. Cauchi*

VIGLIANI, E. C. & FINULLI, M. Osservazioni sulla frequenza dell'influenza da virus A/Asia 1957 nei silicotici. [**Increased Resistance of Silicotics to A/Asian Virus Infection**] *Med. d. Lavoro.* 1959, Aug.–Sept., v. 50, Nos. 8/9, 517–22. [19 refs.] English summary.

Asian virus A influenza was prevalent in Lombardy from September 1957 to April 1958. The authors studied the incidence of clinical infection among silicosis subjects and compared it with that among the rest of the working population of the area. They based their diagnosis of influenza on a pyrexia above 38°C. lasting at least 2 days with headache, pharyngeal congestion and asthenia.

In the Clinica del Lavoro, Milan, there were 5 cases of influenza among 123 silicosis patients, an incidence of 4%, when 100 other (non-silicotic) patients in the same institution produced 20 (20%).

The history of 334 patients who had been admitted for silicosis within the period of 1st September 1957 to 31st March 1958 suggested an infection with Asian influenza in 35 (10·5%), compared with an incidence of 41% in the whole insured working population of 540,985 in Milan Province.

Only 2 of 281 silicotic patients (0·7%) admitted to the Convalescent Home of Villa Giovio, Como, had an attack of Asian influenza, whereas 22 out of the 39 members of the attendant staff (59%) suffered an attack.

The authors also investigated 233 female workers in a Cremona pottery, of whom 68 were affected with silicosis: they found 22 of the 68 (32·4%) to have had an attack of Asian influenza. The incidence among the other 165 workers, who were free from silicosis—was 59%.

The lower incidence of Asian influenza among silicotic patients is statistically significant. The authors compare the increased resistance to Asian virus A shown by silicotic subjects with a somewhat similar behaviour in cases of tuberculosis. They suggest that the quartz which is inhaled stimulates the reticulo-endothelial system with an antibody response to antigenic agents: the quartz would destroy the endothelial cells by which it has been phagocytized, with the liberation of lipopolysaccharides of endotoxin-like properties.

Other workers have shown experimentally in mice the development of increased resistance, following the administration of quartz intravenously, to such bacterial agents as *Escherichia coli* and *Salmonella typhi-murium*. When infected with tuberculosis, guineapigs have been shown to develop a relative resistance to *Bacillus anthracis* and *Brucella suis* [this *Bulletin*, 1957, v. 32, 515]. *J. Cauchi*

KUO, Yü-P'u & LIU, Yin-Tseng. **Silicosis.** *Chinese Med. J.* Peking. 1959, Sept.–Oct., v. 79, Nos. 3/4, 326–35. [19 refs.]

The People's Republic of China celebrated its tenth anniversary on 1st October, 1959. During this 10 years, it is claimed, that under the Chinese Communist Party the country made giant strides in medicine. To mark the occasion the Chinese Medical Journal issued a Commemorative Number (in English) entitled Tenth Anniversary of the People's Republic of China, 1949–1959.

Industrial medicine is represented by a series of 3 papers on silicosis:

I. Neurologic Manifestations of Silicosis Patients.
II. Study of Sense of Taste and Pain Threshold.
III. Study of Vegetative Functions.

All 3 studies are based on the same group of cases and controls: 56 cases of suspected silicosis, 281 cases of silicosis, stages I to III, and 84 cases of silicosis accompanied by tuberculosis; the control group consisted of 116 healthy persons. The diagnosis of the disease was founded on occupational risk and clinical and radiographic findings. No less than 83 patients were aged 20–29, and 213 aged 30–39. In 32 cases the disease developed in under 5 years and in 237 cases between 5 and 10 years. The occupations were hammering (156 cases), pneumatic drilling (159), transportation (56), setting up supports (14), blasting (33) and others (3). [The industry apparently was mining but the nature is not specified.]

The general thesis is that silicosis is a general systemic disease involving the nervous system and other organs in its early development.

Among the symptoms recorded by patients were dizziness and lack of concentration. The authors detected diminution of sense of taste, pain threshold and a sluggish response or loss of the corneal reflex. There was lowering of the vegetative nervous functions as shown by the reaction of skin to ultraviolet radiation, dermographic response, water absorption test and oculo-cardiac reflex. These changes occurred at an early stage of silicosis, indeed sometimes before radiographic evidence of the disease could be identified.

The conclusion is that these findings together with clinical, radiographic and occupational evidence would assist early diagnosis of silicosis.

[The English translation is good. This approach to early diagnosis is entirely new to the reviewer, who hopes that in the future we may have further contributions on pneumoconiosis in China.]

A. Meiklejohn

WORTH, G., GASTHAUS, L., LÜHNING, W., MUYSERS, K., SIEHOFF F. & WERNER, K. Kritische Bemerkungen zur Diagnostik des Lungenemphysems bei Kohlenbergarbeitern. [**Critical Observations on the Diagnosis of Emphysema in Coal Workers**] *Arch. f. Gewerbepath. u. Gewerbehyg.* 1959, v. 17, No. 4, 442–56.

The difficulty of defining and classifying emphysema is well recognized. This paper presents the results of clinical, radiological and physiological indices of the

condition in a group of 1,521 coal-miners with and without silicosis and 240 workers employed in various industries on equally heavy jobs but not exposed to dust.

For their clinical index the authors accepted the classical, if unreliable, physical signs based on shape and mobility of the thorax. The radiological index was determined by the position, contour and movement of the diaphragm and degree of radiolucency of the lung fields on inspiration and expiration. A residual volume of 35% and over of the total lung volume was used as the physiological index.

The results of each index separately and then in combination are given and from the somewhat confused pattern that inevitably emerges the authors conclude that emphysema is commoner in miners without silicosis than in non-miners; that miners with silicosis have more emphysema than miners without and that this is to some degree related to the stage of silicosis.

[Some of the statistical significance tests carried out in coming to these conclusions appear to be invalidated by the different age distributions of the various groups. There are for example no non-miners or miners without silicosis over 60 whereas this age group is well represented in the miners with silicosis.]

I. T. T. Higgins

WHITAKER, Jo A. & VIETTI, Teresa J. **Fluorescence of the Erythrocytes in Lead Poisoning in Children: an Aid to Rapid Diagnosis.** *Pediatrics.* Springfield, Ill. 1959, Nov., v. 24, No. 5, Pt. 1, 734–8.

"In seven children with lead poisoning, a thin wet-preparation of erythrocytes was examined under ultraviolet light. In every case red fluorescence was found in 75 to 100% of the erythrocytes. The fluorescence appears to be related to the amount of free protoporphyrin in the erythrocytes. Over 150 preparations of erythrocytes from patients with a variety of other clinical conditions showed 0 to 50% fluorescent erythrocytes. This test is a simple, rapid, reliable method to aid in the diagnosis of lead poisoning in children."

OLTRAMARE, M., LANG, R. & LE COULTRE, Jaqueline. Sur la numération des hématies à granulations basophiles dans les examens périodiques des ouvriers exposés au plomb. **[Enumeration of Punctate Basophil Cells in Periodical Examinations of Workers exposed to Lead]** *Ztschr. f. Unfallmed. u. Berufskrankh.* 1959, Sept. 15, v. 52, No. 3, 235–42.

In examining punctate basophil cells, which though not pathognomonic of lead poisoning, are particularly numerous and characteristic, the authors classify the granulations, according to their size, as large, medium and fine. They consider punctate cells specific to lead absorption when they number between 1 and 10 per 1,000 erythrocytes. They prefer staining by alkaline methylene blue to May-Grünwald-Giemsa since the former makes nearly twice as many visible as the latter. They also prefer direct light to dark-ground illumination on the grounds that the latter makes it more difficult to distinguish the size of the granulations, which vary with the severity and acuteness of the absorption. Among 50 lead workers, those with no symptoms showed almost entirely fine or medium granulations; in those with slight symptoms of nervousness, constipation, occasional abdominal pain and a haemoglobin level not lower than 80%, some cells contained large granulations; with overt intoxication—anaemia, colic and renal symptoms—the punctate count was never below 8 per 1,000, and coarse granulations were always present.

It is concluded therefore that an increase in the number of punctate cells practically always precedes the appearance of manifest clinical symptoms and that the special value of this enumeration lies in the prevention of the passage of those showing fine and medium granulations to those with coarse.

A typical case is quoted of a workman who felt well and had a haemoglobin level of 85%, but who showed 10·6 per 1,000 punctate basophils, of which 2% had coarse granulations. He was therefore removed immediately from all contact with lead.

In order to reduce the time necessary for counting 10,000 cells, a mathematical calculation of the statistical variation of blood samples has been evolved which it is claimed allows a sufficiently exact estimation for periodical examinations when 4,000 cells are minutely examined; this takes about half an hour, as opposed to more than an hour and a half for the generally accepted minimum number of 10,000.

Ethel Browning

BRUGSCH, H. G. **Fatal Nephropathy during Edathamil Therapy in Lead Poisoning.** *Arch. Indust. Health.* Chicago. 1959, Oct., v. 20, No. 4, 285–92, 1 fig. [41 refs.]

This review deals mainly with the exacerbation of parenchymatous renal disease in chronic lead poisoning which may be caused by Edathamil ($CaNa_2$edathamil, calcium di-sodium versenate). It includes clinical and experimental reports which indicate toxic effects on kidney tubules from various versenate chelating agents, *e.g.* Edathamil after plutonium absorption; edathamil sodium in hypercalcaemia; Versenol, a triacetic acid complex, in haemochromatosis; synergism of Edathamil with cadmium, neither alone causing damage under the experimental conditions; lead versenate or large amounts of Edathamil when given alone. After injection of lead versenate, lead particles accumulate within the excretory part of the kidneys. The lead versenate reaching the kidneys in the chelating drug and the calcium in Edathamil are dissociated probably within the tubules; both the lead and the calcium could damage the tubules and there may be synergism.

Reports from Switzerland [this *Bulletin*, 1957, v. 32, 870, 1171] of progressive fatal renal disease in 4 patients treated with Edathamil for lead poisoning showed tubular necrosis in the proximal tubules and Henle's loops or hyaline casts within the tubules. In one case the lead seemed to have shifted from liver to kidney.

Investigations, if carried out during chelating therapy for lead poisoning, may throw light on the effect of lead on the kidneys, *e.g.* the relation of "free" lead to lead versenate in the tissues; renal clearance of lead versenate; kidney biopsy; blood and urine calcium; and histochemical studies of tissue deposits.

It is recommended that renal function tests should be carried out before, during and after Edathamil therapy; that slow intravenous infusions should be given for not longer than 3 days per course with an interval of at least 2 weeks, and that the daily adult dose should not exceed 2 gm. *M. Patricia Fitzsimons*

Pott, R. Ist die Prophylaxe der Bleikrankheit mit Ca-EDTA möglich? [**Is Ca-EDTA Prophylaxis of Lead Poisoning Possible?**] *Arch. f. Gewerbepath. u. Gewerbehyg.* 1959, v. 17, No. 4, 354–64, 3 figs. [Numerous refs.]

In a copper-lead refinery in Hamburg employing 2,500 workers, about 600 in specially hazardous conditions have been kept under regular clinical and haematological supervision. Although, with one exception in 1953, there have been no cases of severe poisoning, slight disturbances of health have caused absenteeism, and any worker with more than 8 punctate cells in 50 fields, or significant porphyrinuria or both, has been considered a "lead carrier".

In 1957 was begun the use of Ca-EDTA, given orally, in doses of 6 tablets (each of 0·5 gm.) daily to all workers with more than 20 punctate cells in 50 fields and marked porphyrinuria, without interruption of their usual exposure. Estimations of porphyrinuria, punctate basophilia, haemoglobin and blood lead levels, and in some cases urinary lead, were made before and after the treatment.

The urinary lead in 15 out of 35 cases increased during the first 6 days to about 4 mgm. daily, decreasing later. During this time continued exposure was well tolerated, with a diminution of gastro-intestinal disturbance. It is claimed that owing to the more favourable proportion between the possible and actual urinary excretion of lead with oral administration of Ca-EDTA, this method carries less risk than the intravenous of excessive mobilization of lead from bone depots. In the months following the ending of the Ca-EDTA treatment the number of persons with symptoms attributed to lead poisoning increased, but this may have been due to an unavoidable increase in exposure, and it is considered that in these circumstances oral prophylaxis is worth while. Small doses daily (1 to 2 tablets) at the beginning of the shift help to bind the lead absorbed with the dust and respiratory mucus and maintain a slightly increased urinary excretion

Such small doses carry no danger of excessive excretion of trace elements and iron. With regard to possible sensitization to Ca-EDTA and the consequent danger of kidney injury, it is advised that careful choice of the individual workers should be made and that they should be kept under constant medical supervision, with estimations of lead in blood and urine and, where possible, in faeces. *Ethel Browning*

Oltramare, M. La valeur des examens médicaux périodiques pour la prévention du saturnisme dans une fonderie. Essai de classification des sujets suivant le degré d'impregnation par le plomb. [**The Value of Periodic Medical Examinations in the Prevention of Lead Poisoning in a Foundry. An Attempt to Classify the Subjects according to the Degree of Lead Absorption**] *Ztschr. f. Präventivmed.* Zurich. 1959, Sept., v. 4, No. 9, 302–9.

Periodic medical examinations, including basophil granular counts as the main criterion, were undertaken in this brass foundry in Geneva, as some of the measures designed to assess and control the lead hazard when several cases of lead poisoning had suddenly occurred. Production at higher temperatures had been increased, though the same bronze alloy, which contained 25% of lead, had been made for 25 years.

Atmospheric concentrations of lead are shown in a plan to have been well above the MAC and near the furnace to have reached 12 mgm. per m^3. Additional and improved ventilation hoods were installed, a partition was built and the workers were provided with masks to be worn during certain processes.

While improvements were being made and others planned, the periodic examinations and the trend of blood changes assumed great importance. No new cases of poisoning developed. Workers exposed to lead were classified into 5 groups from safe to severely poisoned. A table of the criteria and of the results during the following year shows the improvement in health by the proportions in each group. The critical basophil granular picture, which separated those with slight lead absorption or possible unobtrusive poisoning from those with slight poisoning or evident absorption, was a quantitative or a qualitative one: a total of 5 granular cells per 1,000 red blood cells; or 2 per 1,000 with medium or coarse granules; or 1 per 1,000 with coarse granules. By means of this examination it was possible to pick out those with marked absorption before symptoms appeared. A graph shows the correlation between basophil granulation and lead exposure in a worker after he had returned to work following recovery from severe poisoning. The whole examination is completed at the foundry and the result discussed with the individual and if positive with the management. *M. Patricia Fitzsimons*

Prior, J. T. & Cronk, G. A. **Pathological Changes associated with Aluminium and Zirconium Compounds. An Experimental Study of Skin Pathology following the Application of Aluminium Chlorohydroxide, Aluminium Sulfate, Sodium Aluminium Lactate, and Zirconium Tetraisopropoxide.** *Arch. Dermat.* 1959, Oct., v. 80, No. 4, 447–54, 14 figs.

Observations have been made on the tissue reactions to 2 chemicals used in commercial deodorants—aluminium chlorohydroxide and aluminium sulphate—and two chemicals with deodorant possibilities—sodium aluminium lactate and zirconium tetraisopropoxide. The test chemicals were administered, in doses listed

in the paper, to albino rabbits by 3 routes, subcutaneous, intracutaneous and percutaneous, and were applied to intact undamaged skin and to skin deliberately damaged with superficial lacerations. The reactions from these injections were measured after 24 and 48 hours, 1, 2, 3 and 4 weeks. The diameter of the inflammatory infiltrate was determined and used as an index of the severity of the reaction. Biopsy specimens were taken at intervals to correlate the gross and microscopic findings.

"Acute inflammatory reactions with tissue necrosis and ulceration were noted in association with the subcutaneous and intracutaneous injection of the aluminium compounds. There was a more marked reaction at the injection sites in areas that had been previously injured. Repeated injections, either subcutaneously or intracutaneously, resulted in the same type of cutaneous ulcers, but these were not felt to be more severe than those following the original injections. Granulomas were seen only after the subcutaneous injection of aluminium chlorohydroxide complex. All lesions went on to normal, although delayed, healing. The percutaneous application of these chemical compounds failed to produce any significant lesions.

"The zirconium compound produced significant subcutaneous and intracutaneous reactions only when combined with hexachlorophene. Ulcerative lesions were produced only in areas in which there had been previous injury. Percutaneous application of the zirconium-hexachlorophene mixture produced no changes."

P. J. R. Challen

FRIBERG, L. **Chronic Cadmium Poisoning.** *Arch. Indust. Health.* Chicago. 1959, Nov., v. 20, No. 5, 401–7. [50 refs.]

A review of the literature.

HINE, C. H., DUNLAP, Mary K. & KODAMA, J. K. **Industrial Toxicology. I. General Principles and New Developments.** *Arch. Intern. Med.* 1959, Nov., v. 104, No. 5, 816–26. [42 refs.]

MAY, J. Die quantitative Messung von Lösemitteldampf in Luft. [**The Quantitative Estimation of Vapour Concentrations of Solvents in Air**] *Staub.* Düsseldorf. 1959, Nov. 1, v. 19, No. 11, 387–91, 2 figs.

The English summary appended to the paper is as follows:—

"The article represents the operating regulations for two methods for the quantitative measuring of vapor concentrations of solvents in the air—regulations as required for the protection of workers. The advantages and disadvantages of a gravimetrical method, by which the solvent is adsorbed to active carbon, are compared with the advantages and disadvantages of the interferometrical measurement by the use of a 1 m-chamber laboratory gas interferometer. With the interferometer it is possible to measure exactly down to very small vapor concentrations of solvents of known or unknown composition in air. There resulted from several diluents currently used in industry as smallest measurable concentration a mean value of 0·05 mg/l or 10 p.p.m. respectively. Attention is drawn to the great advantage of an extremely rapid approximative quantitative measurement."

IANNACCONE, A. & CICCHELLA, G. **Histochemical Alterations of the Endocrine Glands in Experimental Benzene Intoxication.** *Scientia Med. Italica.* 1959, July–Sept., v. 8, No. 1, 59–77, 13 figs. [20 refs.]

Several plates, mostly in colour, of slides of endocrine gland preparations illustrate the histological appearances which, with staining techniques, are described in some detail. Sections were made of the pituitary, adrenals, thyroid, testis, thymus, parathyroids and pancreas—and intestinal mucosa—from 14 rats killed on the day after 22 daily subcutaneous injections of 1 ml. per kgm. of a solution containing equal volumes of benzene and olive oil, and from 4 control rats after 1 ml. per kgm. of olive oil similarly administered.

Functional changes which the alterations in cell structure could signify are discussed and certain conclusions are proposed. Changes in some of the pituitary cells, implying increased secretion of specific hormones, were reflected in changes in the thyroid and adrenal cortex which indicated hyperfunction of these glands, and in the pancreas which indicated some variably increased activity. Other pituitary cells showed features which suggested that their hormones were not released, and this would explain the depressed maturation of the germinal epithelium which was observed in the testis. Administration of benzene to the rats had evidently been followed by endocrine alterations similar to those described in animals exposed to various types of stress. This view was supported by changes in the adrenal medulla which showed signs of enhanced activity, and in the thymus of slight involution.

The intestinal mucosa was examined to find out whether benzene exerted any direct anti-mitotic effect independently of any pituitary and adrenocortical reaction. This was investigated because of a previous finding of inhibition of erythroblast mitosis in a patient with acute benzene erythraemia, and also because in the poisoned rats increased numbers of mitoses were found in the testis and thymus. However, no abnormalities were detected. Mitoses in Leiberkühn's crypts were quantitatively and qualitatively similar in poisoned and control animals.

M. Patricia Fitzsimons

INDIA, GOVERNMENT OF: MINISTRY OF LABOUR & EMPLOYMENT. **Survey of Carbon Disulphide, Hydrogen Sulphide and Sulphur Dioxide Hazards in the Viscose Rayon Industry in India.** 59 pp. [68 refs.] 1959. Report No. 18. New Delhi: Office of the Chief Adviser Factories.

In India there are 5 factories engaged in the manufacture of rayon, and in 4 of these the viscose process is used. The investigation described in this report was carried out in 3 of the viscose factories, for the fourth came into operation only recently.

Observations in various parts of the carbon disulphide plants in these factories showed that in nearly all instances the concentration of sulphur dioxide exceeded the accepted maximum allowable concentration of 10 parts per million of air, and often substantially. At 2 of the factories sulphuric acid is manufactured to meet factory requirements, and in these sulphuric acid plants the concentrations of SO_2 in the general atmosphere were below the limit of 10 parts per million.

Generally, in the atmospheres of the workrooms in the viscose sections, the combined concentrations of hydrogen sulphide and carbon disulphide did not exceed 20 parts per million, but these gases were present in toxic quantities at 2 factories during the operations of cleaning churns and discharging xanthate from the churns into the mixers. It appeared that in the spinning sections the gases liberated from the spinning baths were not properly exhausted, for excessive concentrations of H_2S and CS_2 were frequently found. In all the factories the CS_2 retort attendants were exposed to excessive concentrations of SO_2.

Medical examinations were made of 270 workers out of the 1,400 who were exposed to the toxic gases. The signs and symptoms noted have been grouped as digestive, mental, neurological, and ophthalmological, and blood counts were also made. In a series of statistical tables the frequency of occurrence of the various types of signs and symptoms is shown. It is said that the frequency is much less than has been reported for viscose workers in other countries.

From the blood studies it was found that 44% of those examined showed gross anaemia (less than 3·5 million red cells per cmm.), and that 25% showed leucopenia (less than 5,000 white cells per cmm.).

Of the workers examined 27% were diagnosed as showing effects of CS_2, 10% as being affected by H_2S, and 9·5% by SO_2. The highest incidence of CS_2 intoxication was among those employed in the spinning departments. Of the 20 churn-room workers in viscose departments who were examined, 7 were affected by CS_2.

Of the 270 workers examined, 192 were exposed to CS_2. Of these, 139 were exposed to concentrations of CS_2 not exceeding 20 parts per million of air, and 53 to concentrations ranging from 21 to 60 parts per million, but usually between 21 and 40 parts per million. Of the first group 27% showed some degree of poisoning, while of the second group 49% were affected.

On the basis of the knowledge acquired during the investigation recommendations are made as to the precautions which should be taken to safeguard the health of the workers in the industry. *Thomas Bedford*

HENSON, E. V. **Toxicology of some Aliphatic Ketones.** *J. Occup. Med.* Chicago. 1959, Nov., v. 1, No. 11, 607–13. [28 refs.]

"The most important physiological response to the inhalation of any of the ketones is narcosis. In the industrial application of these materials, this anesthetic effect is usually not observed; for, with concentrations at which this could occur, the ketones give adequate warning by odor and irritation of the conjunctiva and mucous membranes of the upper respiratory tract. There is sufficient evidence to show that the narcotic and irritating effects and toxicities of the ketones increase with an increase of molecular weight. Their solvent action on the skin is not of primary importance but may be conducive to secondary changes by sensitizing agents and infections.

"The absence of reports of intoxications by ketones despite the enormous volumes used industrially indicate that the low order of toxicity of these substances for experimental animals holds as well for man."

SAVICEVIC, M., MILIJIC, B. & STANKOVIC, M. Contribution à l'étude de l'intoxication chronique par l'oxyde de carbone. **[Study of Chronic Carbon Monoxide Poisoning]** *Acta Med. Iugoslavica.* 1959, v. 13, No. 2, 129–37. [22 refs.]

The problem of chronic poisoning due to carbon monoxide has been discussed for many years, and there are still differences of opinion as to whether or not such a condition exists. Since many industrial workers are employed in conditions where the concentration of CO in the atmosphere exceeds what is regarded as the maximum allowable concentration the problem is of considerable practical interest. In this paper the authors describe an investigation carried out on persons whose work was done in atmospheres in which the concentration of CO varied up to 300 parts per million, the concentration being most frequently between 50 and 140 parts per million.

In addition to measurements of the atmospheric environment, including the concentration of CO, clinical and laboratory examinations were made. The clinical examinations were carried out by a neurologist and a specialist in industrial medicine. The laboratory tests included blood examinations, including the determination of CO in blood, urine analysis, electrocardiographic examination, and the determination of basal metabolism. 153 persons were examined, with many re-examinations, and in addition systematic examinations were made of a control group of persons of similar age and economic status, who worked in similar environmental conditions with the exception that there was no CO hazard. The numerical results of these examinations are tabulated in the paper.

From the results described the authors conclude that there are clear indications of chronic CO poisoning, but that there are no particular pathological signs of it. The similarity of the subjective symptoms and the objective signs which characterize this intoxication with the manifestations of neurosis makes it necessary to exercise prudence, and chronic CO poisoning should only be diagnosed after several careful clinical examinations and laboratory tests. In this connexion a blood CO concentration of more than 0·75% coinciding with the presence of more than 50 parts of CO per million of air in the atmosphere of the workplace, and the occurrence of asthenia, headache and vertigo (and especially if before employment in that place the worker was free of those symptoms) indicates that one should take radical steps to eliminate the CO hazard. If there is

more than 0·75% of CO in the blood of a worker exposed to risk he should be transferred to a less dangerous place. *Thomas Bedford*

HOCHWEBER, M. Farben und Lacke, die Werkstoffe des Malergewerbes. **[Pigments and Lacquers, the Basic Ingredients of the Paint Industry]** *Ztschr. f. Unfallmed. u. Berufskrankh.* 1959, Sept. 15, v. 52, No. 3, 197–217.

Modern painting operations require low-boiling, rapidly volatile, potent solvents with components for preventing formation of film, pigments, binding materials, solvents and thinners, adhesives, emulsifiers, and disinfectants. The author describes the various pigments.

Solvents are usually mixtures of aliphatic and aromatic hydrocarbons; cyclohexane, methylcyclohexane, tetralin, decalin, alcohols, ethers and esters, and the nitroparaffins have now to some extent replaced the once ubiquitous turpentine. Glycol and furfuran derivatives are much used as lacquer solvents.

Binding materials have undergone much development during the last 30 years with the introduction of nitrocellulose lacquers and of synthetic resins obtained by polymerization; isocyanates and epoxy compounds and silicones are the most recent examples of this development.

Plasticizers such as phthalates, tricresyl phosphate and chlorinated diphenyls are added to lacquers as softening agents. Paint strippers include methylene chloride, esters, ketones, aliphatic and aromatic hydrocarbons, including benzol and its homologues. Fluorine compounds are often used as a preliminary treatment, and phosphoric acid as a rust remover. The chief materials used for fire-proofing wood are diammonium phosphate and borax.

The main hazards from all these materials are summarized according to the processes in which they are used. Scraping of old paint involves inhalation of lead compounds and burning paint involves inhalation of zinc fume and decomposition products such as acrolein. Paint stripping with alkalis causes skin contact and in stripping with mixed solvents, both inhalation and skin contact occur. Sand spraying with quartz can cause silicosis. Chemical derusting and fluorizing bring contact with phosphoric and hydrofluoric acid and its salts.

All methods of paint application involve inhalation of solvent vapours and volatile components; spraying carries the additional hazard of fine pigment particles, and hand cleansing with thinners, the risk of de-fatting of the skin. *Ethel Browning*

BOSSHARD, R. Die gewerbemedizinischen Aspekte der beruflichen Gefahren im Malergewerbe. **[The Industrial Medical Aspects of Occupational Hazards in the Paint Industry]** *Ztschr. f. Unfallmed. u. Berufskrankh.* 1959, Sept. 15, v. 52, No. 3, 218–34. [11 refs.]

The health disorders most characteristic of painters are classified as external (skin contact) and internal (absorption of solvents, thinners, pigments, resins and strippers).

An analysis is made of 600 cases of illness occurring among painters between 1951 and 1957. About ¾ of these were cases of eczema, of which ½ were due to turpentine, ⅓ to other thinners, pigments and lacquers, and of the remainder Desmodur was responsible for nearly as many as solvents.

Eczema and skin affections are becoming increasingly common. The allergic form is observed chiefly with turpentine, and to some extent with solvents, thinners and resins. The toxic form, due to direct chemical action on the skin, is associated predominantly with thinners, soaps and alkalis. A further group includes the "defatted" skin, susceptible to infection, chemical contacts and sensitization, and due mainly to washing the hands frequently with unsuitable materials such as thinners—turpentine etc.

"Internal" poisoning is associated chiefly with solvents, especially the volatile lacquer solvents, which are particularly harmful when applied by spraying. Acetates are potent irritants of respiratory mucous membranes. Many thinners contain toluol and xylol, which the author considers responsible for the "prenarcotic syndrome" of headache, drowsiness, giddiness, palpitation, nausea, gastric pain, lassitude, sleep disturbance, general irritability and intolerance to alcohol. Sometimes the pigment spray is responsible for additional respiratory irritation. 2 cases of this combination of respiratory and "prenarcotic" disorder are described. One, a worker employed in painting the outside of a vessel containing heated alkali, the paint being a mixture of synthetic resin with toluol, ketones and acetates, is described as a case of upper respiratory irritation with "slight toxic encephalosis", the latter attributed to toluol. The other, a man employed in spraying radiators and shutters, showed in addition to gastro-intestinal disturbance, pharyngitis and cough, some anaemia and leucopenia. It was not established whether benzol was a constituent of the spray, or whether toluol and xylol cause similar blood changes, though one subject exposed to a toluol-xylol mixture showed a leucocyte count of 3,400 per c.mm. One exceptional case of polyradiculitis occurred in a man using a rust remover containing a toluol-xylol mixture.

The author quotes cases of a blood picture similar to that of benzol poisoning reported by BÄTTIG *et al.* (*Arch. f. Gewerbepath. u. Gewerbehyg.*, 1958, v. 16, 555) in 1955 from the use of trimethyl benzol [see also this *Bulletin*, 1957, v. 32, 160].

Lead poisoning from actual painting is now rare but still occurs in processes where paint is removed by scraping and burning in unhygienic conditions, and an increasing risk is that of silicosis from sand-blasting.

Zinc fever may arise from the use of zinc-containing lacquers or the application of molten zinc to wire.

Isocyanates, especially Desmodur T, constitute a special danger in the newer lacquers. Desmodur T is a mixture of meta- and para-diisocyanate, but modifications of the Desmodur lacquers are now being made with a reduction of the volatile component.

The subjective and objective symptoms of 81 cases due to isocyanates are presented in tabular form; the most frequent subjective symptom (93%) was a feeling of constriction in the chest; the most frequent observation tracheitis and bronchitis (81%), followed by

"asthma" (60%). None of these cases was severe, the majority recovering within 2–3 weeks; they were regarded as an "acute toxic" injury rather than an allergic reaction as postulated by many authorities.

It is concluded that among the complex risks of this industry, the most important preventive measure against inhalation is the provision of adequate ventilation or protective respiratory apparatus, and against eczema, avoidance of all unnecessary contact and use of protective creams to avoid too frequent washing of the hands. *Ethel Browning*

MINISTRY OF LABOUR & NATIONAL SERVICE. H.M. FACTORY INSPECTORATE. **Methods for the Detection of Toxic Substances in Air. Booklet No. 6. Carbon Bisulphide Vapour.** 2nd Edition. 10 pp., 1 diagram. 1959. London: H.M. Stationery Office. [1s. 3d.]

The contents are essentially the same as before, when the last edition was published by the Department of Scientific and Industrial Research in 1939 [this *Bulletin*, 1939, v. 14, 799]. The few general amendments are similar to those indicated in the notes above on the new booklet on aniline [see below]. The section on the poisonous effects of carbon bisulphide has been rearranged.

Concentrations in the atmosphere of carbon bisulphide in excess of 20 p.p.m. (v/v) indicate unsatisfactory working conditions (threshold limit value for continued exposure, American Conference of Governmental Industrial Hygienists, 1958). A concentration of 500 p.p.m. is likely to induce severe toxic effects after exposure for 1 hour.

The test, as before, depends on comparing with a series of standards the coloured solution which results when carbon bisulphide vapour is pumped into a mixed reagent which contains ethylamine, which had been dissolved in benzene, and copper acetate which had been dissolved in absolute alcohol. The coloured compound is copper diethyldithiocarbamate. The 4 standards are prepared just before the test from the reagents and dilute alcoholic solutions of carbon bisulphide of known strengths. A lead acetate filter paper at the pump inlet removes traces of hydrogen sulphide. The concentration is estimated from a table which sets forth values for concentrations of carbon bisulphide lying between 8 and 1,320 p.p.m.

First-aid advice is limited to the generalization that the principles are those applicable to other narcotic vapours: reference should be made to Form 395—Dangers from Gassing and Burns, H.M.S.O., for details. *M. Patricia Fitzsimons*

MINISTRY OF LABOUR & NATIONAL SERVICE. H.M. FACTORY INSPECTORATE. **Methods for the Detection of Toxic Substances in Air. Booklet No. 11. Aniline Vapour.** 2nd edition. 10 pp., 1 diagram. 1959. London: H.M. Stationery Office. [1s. 3d.]

The method has not been altered. The contents remain essentially the same as when published in 1939 by the Department of Scientific and Industrial Research following discussions begun in 1932 by the Association of British Chemical Manufacturers with the Home Office. The Factories Acts (FA) of 1937 and 1948 have extended the use of the test which was one of a series planned to help the chemical industry to comply with the Chemical Works Regulations (CWR), 1922. Among the few amendments are the following.

In testing an enclosed space where the concentration may rise, no person should enter the space unless he is wearing breathing apparatus as required under Section 27 of the FA 1937 and Regulation 7 of the CWR. Valves of the standard hand-pump should be renewed if found defective during the test necessary before use; wrapping material, *e.g.* Cellophane is a satisfactory material for them.

Concentrations of aniline vapour above 5 p.p.m. by volume in the atmosphere indicate unsatisfactory working conditions (threshold limit value, American Conference of Government Industrial Hygienists, 1958). Continual exposure to 20 p.p.m. causes gradual poisoning. Exposure to 100 p.p.m. leads to severe toxic effects in 1 hour. The description of the poisonous effects makes the point that cyanosis may be missed in coloured people or in certain artificial illumination.

First aid instructions give greater emphasis to changing clothing contaminated by liquid or vapour, to washing affected skin with cool water and to keeping the casualty at rest.

In the test, as before, aniline vapour is pumped into dilute hydrochloric acid; bleaching powder solution is added and afterwards the phenol and ammonia reagent. The permanent blue colour produced is matched against 5 standards prepared from Toluidine Blue. A table gives 8 concentrations between 5 and 200 p.p.m., each corresponding to the standard matched and the number of strokes of the pump used. *M. Patricia Fizsimons*

SCHÜTZ, E. Massnahmen zur Verhinderung percutaner Anilin- und Phenol-Vergiftungen. [**Measures for the Prevention of Percutaneous Poisoning with Phenol and Aniline**] *Berufsdermatosen.* Aulendorf. 1959, Oct., v. 7, No. 5, 266–74, 2 figs. [19 refs.]

The greatest hazard of poisoning from skin contamination arises from phenols, aniline compounds, nitrobenzene and some plant insecticides. A table lists the substances recommended and not recommended for cleansing the skin from phenol and aniline. Animal experiments were carried out to determine which could be regarded as fulfilling the criteria of being non-toxic in themselves, capable of dissolving, emulsifying and facilitating the removal by washing, and of not furthering absorption of the toxic compounds by the skin.

The substances tested were applied to the shaved abdominal skin, both intact and abraded, of rats, after preliminary application of phenol and aniline in lethal doses. For phenol, the substances tested were water, ethyl alcohol, glycerin, olive oil and a polyethylene glycol; for aniline, the same with the exception of glycerin and olive oil.

With phenol, only the glycol compound had an

ameliorating effect on the symptoms of poisoning when applied to injured as well as intact skin; 40% ethyl alcohol had a protective effect with injured but not with intact skin; glycerin and olive oil were both practically ineffective. With aniline also the polyethylene glycol gave the greatest protection; ethyl alcohol prevented the death of 3 out of 5 rats with unbroken skin but was ineffective when the skin was injured; tap water was quite ineffective.

Ethel Browning

PFITZER, E. A. & SEALS, J. M. **Determination of Decaborane with Dipyridyl Ethylene.** *Amer. Indust. Hyg. Ass. J.* 1959, Oct., v. 20, No. 5, 392–5, 4 figs. [23 refs.]

BEHRBOHM, P. & BRANDT, B. Über allergische und toxische Hautschäden bei der Herstellung und Verarbeitung von Hexachlorcyclohexan. [**Allergic and Toxic Skin Injuries in the Production and Use of Benzene Hexachloride**] *Arch. f. Gewerbepath. u. Gewerbehyg.* 1959, v. 17, No. 4, 365–83, 2 figs. [Numerous refs.]

Crude benzenehexachloride (BHC) is a mixture of isomers. It can be absorbed by the skin, gastrointestinal canal and by inhalation.

Skin injuries in man have been almost exclusively observed in workers employed in its production. The authors have investigated 26 cases, some described as "probably allergic" and some as "probably toxic" eczema. The allergic cases occurred especially in the department where the gamma isomer is obtained from the crude product; the majority of the toxic cases where a mixed insecticide known as "cotton dust" (DDT, BHC, sulphur and a filler) was handled, particularly during the summer months. The lesions were localized especially in folds of the skin, such as the elbows, where sweating was most marked. This is attributed to the decomposition of BHC by the alkaline sweat; it is not known which component was responsible, but it is assumed, on the evidence of skin testing reactions, that cotton dust preparations are more toxic than Lindane and that the impurities in technical BHC are more easily attacked chemically than BHC itself.

Allergic eczema was partly associated with the severity and duration of the first attack; it sometimes recurred after an interval away from work when clothes which smelt of technical BHC were re-worn. Patch tests showed no positive reaction to pure alpha, beta, gamma and delta isomers but increased with increasing grades of impurity, suggesting, in contrast to other observations—including those of the American Committee on Pesticides in 1951 [this *Bulletin*, 1952, v. 27, 452]—that the cause of the allergic contact eczema is not the gamma or delta isomer but the impurities in the so-called "benzine layer" formed during the crystallization of the technical product.

Protective measures which have greatly reduced the incidence of severe contact eczema are avoidance of the production of cotton dust during the warm season; powdering with talcum after bathing: frequent changes of clothing and increased removal of dust by exhaust ventilation. Allergic dermatoses are to be expected only with the use of impure preparations of BHC.

Ethel Browing

KLUGMAN, H. B. **Parathion Poisoning.** *South African Med. J.* 1959, Oct. 24, v. 33, No. 43, 899–901. [13 refs.]

This is an account of 4 cases of accidental poisoning by parathion; the patients were members of a family who had eaten a contaminated stew.

The author discusses modern work on this condition and advocates the following system of treatment (in which pyridine-2-aldoxime methiodide (P2-AM) is used) recommended by GROB and JOHNS [this *Bulletin*, 1958, v. 33, 768]:—

"1. Terminate exposure by removal from site of poisoning, removal of contaminated clothing, copious washing of skin and eyes, and gastric lavage.

"2. Maintain a patent air-way by endotracheal intubation or tracheotomy if necessary.

"3. Apply artificial respiration manually or by the use of the poliomat, artificial lung, or bellows on closed-circuit anaesthetic machine.

"4. Administer atropine, 2–4 mg. (1/30–1/15 gr.) intravenously, followed by 2 mg. every 3–8 minutes until muscarine-like symptoms disappear, and whenever they reappear; a total of 24–48 mg. may be required the first day.

"In less severe intoxication give 2 mg. intravenously or intramuscularly, and repeat at 20-minute intervals until muscarine-like symptoms are relieved; maintain a mild degree of atropinization for 24–48 hours.

"5. In severe intoxication give 2,000 mg. of 2PAM [P2-AM] (500 mg. per minute) intravenously, and repeat the dose if weakness is not relieved or recurs. In moderate intoxication give 1,000 mg. intravenously and repeat if necessary.

"6. Alleviate conclusions by anaesthesia if necessary."

Charles Wilcocks

IPPEN, H. Porphyria cutanea tarda und Beruf. [**Porphyria cutanea tarda and Occupation**] *Berufsdermatosen.* Aulendorf. 1959, Oct., v. 7, No. 5, 256–66, 1 fig. [85 refs.]

The rising incidence of cutaneous prophyria in later life has aroused the suspicion that exogenous as well as congenital factors may play some part in its development.

A survey of 168 published cases, with a classification of their various occupations, indicates the possible aetiology of alcohol, exposure to light and to lead, in 80%. Among the lead group the most important single class of workers (11·9%) were drivers of automobiles, with an exposure to lead benzine.

Many observers regard the liver as the site of disturbance of prophyrin metabolism, fatty degeneration and cirrhosis being incriminated as the cause of an increased excretion of porphyrin, which may cause

pigmentation of the skin. Many cases, in the absence of a high degree of porphyrinuria, have been regarded as due to haemachromatosis, but have occurred after many years of exposure to lead.

The author describes the case of a man aged 65 in whom the typical skin lesions of late porphyria occurred after 20 years as an electric welder and 14 years as a repairer and welder of lead-containing tools, during which period he was exposed to lead vapour. He had no clinical symptoms of lead poisoning, but a higher than normal excretion of copro- and uroporphyrin; the bone marrow showed increased erythropoiesis and laporoscopy of the liver haemosiderotic fatty cirrhosis. There was also an increased sensitivity to ultraviolet light. It was concluded that the exposure to lead and to ultraviolet light had been determining factors.

Porphyria cutanea tarda seldom causes an incapacity for work greater than 30%, but in rare cases lesions resembling sclerodactyly may follow.

A close association between cutaneous porphyria and many industrial conditions is difficult to demonstrate, but the author believes lead to be the most important underlying factor. He recommends that in such cases there should be a change of occupation, and in all cases abstention from alcohol. *Ethel Browning*

JANSSON, H. & SCHULTHEISS, E. Überempfindlichkeit gegenüber Dioxyphenylhexan (= Hexylresorzin). **[Hypersensitivity to Dioxyphenyl hexane (= Hexylresorcinol)]** *Berufsdermatosen.* Aulendorf. 1959, Oct., v. 7, No. 5, 287–90.

2 cases are described of eczema of the foot following the application of an anti-mycotic ointment to the spaces between the toes. In one case the eczematous lesions were confined to the dorsum of the foot, in the other there were also isolated patches on the limbs. Patch tests with the ointment gave positive reactions in both cases while the ointment base alone was negative. With the addition of Chlorcarvacrol and the propyl and benzyl esters of *p*-hydroxybenzoic acid the results were also negative, but the addition of dioxyphenyl hexane gave a positive reaction. Further tests were made with a large number of substances, including resorcin, to determine whether the hypersensitiveness in these cases was specific to hexylresorcinol or a general allergic skin condition. In the first case the only weakly positive test was obtained from a 50% tar ointment with a Eucerin base.

In the second, there was a positive reaction to several of the substances tested, including potassium dichromate, pyrogallol, formaldehyde and resorcin. This indicated that the first case was one of specific allergy to hexyl resorcinol, while the second was one of polyvalent allergy.

A further series of tests is contemplated, with a larger number of substances and a wider range of subjects, both healthy and eczematous, with a view to estimating the present incidence of hypersensitivity to hexyl resorcinol, and the possibility of an increasing number of cases of hypersensitivity to substances with which resorcin is combined. *Ethel Browning*

KRÖBER, Fritz. **Beruflich bedingte Haut-Tuberkulosen. Übersicht unter Bezug auf die Literatur der Jahre 1930–1957.** *Berufsdermatosen.* Monograph Vol. 2 [Occupational Tuberculosis of the Skin], 62 pp. 1958. Aulendorf i Württ: Edito Cantor, Germany. [DM.6.20.]

The object of this monograph is to provide a comprehensive and, as far as possible, exhaustive account of tuberculosis of the skin as an industrial or occupational disease. It is based on extensive clinical experience and the bibliography contains almost 300 references.

Those who are concerned with giving opinions on whether or not a disease is due to the patient's occupation or who administer social and industrial insurance schemes by which workers benefit when incapacitated by industrial disease often find it difficult to decide whether tuberculosis of the skin (and indeed any form of tuberculosis) is occupational. The author has endeavoured to resolve some of the difficulties which arise in this connexion.

As the age at which primary tuberculous infection occurs is rising, primary infection of the skin is no longer seen most commonly in children but in older persons in the 2nd and 3rd decades of life *i.e.* in workers.

Many examples of primary infection of the skin are given: in medical and nursing personnel, veterinary and other animal workers and butchers. Ritual circumcision of infants and sexual intercourse have been followed by localized genital tuberculosis, and infection of the feet in swimming baths has been reported.

Post-primary infection of the skin may be exogenous or endogenous, and exogenous infection can be divided into that following reinfection (when the primary lesion has completely healed) and superinfection when existing tuberculous infection persists.

It may be extremely difficult and even impossible to distinguish between a primary skin complex and exogenous reinfection. X-ray examination of the lungs, abdomen and peripheral lymph nodes may help by showing calcification, and in some cases the history of the patient's environment may assist greatly. Exogenous superinfection of the skin is still harder to diagnose in the presence of an active tuberculous process elsewhere in the body because of uncertainty whether the skin lesion is autogenous or exogenous.

Tuberculous verrucosis cutis, exogenous lupus vulgaris (inoculation lupus), scrofuloderma and tuberculous ulceration of the skin are described with examples of their occurrence.

Tuberculosis of the skin may follow tuberculosis in another part of the body, the already existing disease having been accepted as occupational, or it may be due to occupational trauma in a person whose existing tuberculosis has not been accepted as occupational. The difficulties in assessing such cases are presented in much detail and examples of various combinations given.

The relationship of various animals to tuberculosis of the skin in man is discussed. A useful table shows the pathogenicity of different types of the tubercle bacillus for man and many other animals and birds.

The final chapter relates the assessment of skin tuberculosis to various aspects of resistance, allergy, race, sex and age and the condition itself to other diseases. *H. G. Calwell*

HARRIS, W. B., BRESLIN, A. J., GLAUBERMAN, H. & WEINSTEIN, M. S. **Environmental Hazards associated with the Milling of Uranium Ore. A Summary Report.** *Arch. Indust. Health.* Chicago. 1959, Nov., v. 20, No. 5, 365–82, 4 figs.

Suggestions for lessening occupational exposure conclude this report from the Health and Safety Laboratory of the U.S. Atomic Energy Commission. Among industries which entail handling and processing radio-active materials, the mining and milling of uranium ore present a growing hazard to large industrial populations. Regulations sometimes demand time-consuming analyses. Some of the objectives of this elaborate study were to assess hazards and present-day control measures inside and outside the plants, including the proportion of hazard arising from individual isotopic components, in order to devise, if possible, simple and trustworthy procedures.

Extraction of the concentrate, an alkaline uranate salt, is illustrated by a flow sheet of basic processes. In 12 mills processes were studied. Air samples were measured for α-emitting dust, its composition, particle size and silica and vanadium content. Air-dust samples, after being counted for gross activity, were analysed for uranium, and radium was separated and counted after a 30-day delay to allow daughters to build up; the radio-activity attributable to these components was compared with the original to determine how the values corresponded with theoretical radio-active equilibrium. Plant effluents were analysed in the same way but further study is necessary. Radiation measurements were made inside and outside. Air over tailings piles was analysed for radon. Urinary uranium excretion rates were measured. 16 tables of results are given. In evaluating the dust hazard the total α-count was useful and it may not always be necessary to count isotopes, but difficulties of interpretation and of timing show that a new method, now being investigated, would be valuable.

Recommendations include better house-keeping, covering, wet-working, local exhaust, vaccum or wet cleaning and, for various processes, isolation, respiratory protection or personal monitoring equipment. Education of workers is advised. Discharge of solid waste to local ground water should be limited. The general public, particularly children, should not be permitted near ore heaps or tailings materials lying outside the plants. [This article should be read in full.]
M. Patricia Fitzsimons

DALHAMN, T. & HOLMA, B. **Animal Experiments on the Spread of Inhaled Radioactive Red Phosphorus in the Body.** *Arch. Indust. Health.* Chicago. 1959, Nov., v. 20, No. 5, 429–31, 2 figs.

"A study of the deposition and retention of P^{32}-labeled red phosphorus of mean particle diameter of 0.46μ inhaled for one hour at 5 mg. per cubic meter by mice indicated by the technique of autoradiography that the phosphor was confined to the upper respiratory tract and the gastro-intestinal tract but was retained in the lungs for at least 10 days."

ECKARDT, R. E. [M.D., Ph.D., F.A.C.P.] **Industrial Carcinogens.** pp. xi+164, 43 figs. 1959. New York 16: Grune & Stratton, Inc., 381 Fourth Avenue. [46s.]

Eckardt describes this book as a "starting point for the student" but it is more than this. It is a balanced impartial account of all the important aspects of industrial carcinogens, though necessarily incomplete in view of its relatively small size and of the existing gaps in the knowledge of carcinogenesis.

The historical introduction is of great interest, beginning with Sir Percivall Pott's description of chimney sweep's cancer in 1778, and depicting the gradual recognition of lung cancer in the Schneeberg miners and in chromate and asbestos workers, bladder cancer in dye workers, bone sarcoma in radium dial painters, to the nasal sinus cancers reported in 1952 in workers in the manufacture of isopropyl alcohol.

In spite of the many strong presumptive evidences of a close association of certain cancers with certain occupational exposures, Eckardt gives a timely warning against allowing these to disturb the proper perspective of this controversial subject. He gives a long section on animal experiments, without which progress must have been much slower, noting that there are many pitfalls to be avoided in attempting to apply their results to man or even to different animal species.

The next section is on occupational cancers, over 75% of which have been those of the skin, but neither these nor cancers of other parts of the body have specific characteristics which differentiate them from those of "unknown" origin. It is therefore of great value to be given information which will be helpful in deciding whether a particular cancer is or is not associated with the sufferer's work. The location of the growth on a site which has been subject to contact with a known or suspected carcinogen, pigmentary changes of the skin exposed to coal tar, the predilection of the trigone of the bladder for occupational bladder cancer; such facts and a careful review of the clinical features and the nature of the occupational exposure, are indispensable in reaching a decision. A substantial section is devoted to protective programmes, and shorter sections to medico-legal considerations and speculations about the future.

To every research student who contemplates devoting himself to this field of experiment this book, while serving as a warning against unjustified enthusiasm, must be an inspiration in his search for knowledge and a final rewarding result. *Ethel Browning*

HEATING, LIGHTING AND VENTILATION

ANGUS, T. C. **Air Conditions in Operating Theatres. Part I. A Study of Requirements and of Mechanisms, for the Control of Air Temperatures and Air-Borne Bacteria.** *Med. Press.* 1959, Aug. 5, 113–16. **Part II. Air-Borne Bacteria.** *Ibid.*, Aug. 12, 134–8, 1 fig. [17 refs.]

Some 20 years ago it was the practice to require temperatures of up to 80°F. or so in operating theatres, but at the present time the tendency is to maintain considerably lower temperatures. The author quotes various opinions as to the temperature that is desirable for the patient, and reaches the conclusion that in general a temperature of between 60° and 70°F. is now appreciated, instead of the much higher temperature required formerly. Yet on account of the hibernation techniques that are sometimes needed and the fact that some operations may still require high temperatures, it is desirable that any temperature between 60° and 80°F. should be obtainable at will. Hence it is now necessary to consider the need for keeping operating theatres cool on hot days.

It is desirable that the relative humidity in a theatre should be kept at between 55 and 60%. Various rates of fresh air supply have been used, but 10 air changes an hour is a commonly accepted figure.

After discussing methods of cooling and the control of airborne bacteria, the author outlines a design for air conditioning which should incorporate most of the agreed requirements and allow of such variations that experience may show to be desirable. The filtered and conditioned air would be admitted through adjustable louvres set in the walls at a height of about 8 feet from the floor, the direction and velocity of the entering air being controllable to the surgeon's requirements. A small proportion of the supply air might be used to form a clean-air curtain around the patient. Sterility of the air supplied for the curtain could be further assured by passing that air over ultra-violet lamps placed within the delivery duct. Air would be removed from the theatre through exhaust grilles at low level, including some in the floor near the operating table, and through a hood placed over the sterilizers. To ensure that no blow-back through the exhaust ducts could occur the ducts should be positively shut off by motor- or solenoid-operated dampers if the plenum fans stop.

It has been suggested that to protect the patient from bacteria-laden air, and the surgeon from anaesthetics, the ventilation of the theatre should be by the uniform downward displacement of filtered air from ceiling to floor, but the author names two objections to this. The air speed would have to be so low that the downward movement of air would be counteracted and overcome by local convection currents and by movements of people, and the staff would suffer from the thermal disability of working in stagnant air. In hot weather when cool air is injected there is reason to believe an unstable condition of air flow would result from the cold air being admitted at the top of the room.

Two questions which remain to be answered are: (1) whether the installation of refrigerating plant is essential for air conditioning British operating theatres in summer, and (2) with what efficiency, in terms of particle size, should the filtration of the ventilating air be carried out. *Thomas Bedford*

Food and Nutrition

The order in this section is:—**General; Foodstuffs; Food Poisoning.**

GENERAL

BROCK, J. F. **Nutrition and the Clinician.** *Lancet.* 1959, Nov. 21 & 28, 859–64; 923–7, 2 figs. [Numerous refs.]

These two Rolleston lectures delivered to the Royal College of Physicians of London range widely over their theme. The contributions of nature and nurture to the multiple aetiology of human disease are first discussed in general terms. The geographical pathology of nutritional diseases is then mentioned. Cape Town has provided the author with a ready made laboratory for studying diseases associated with inadequate diets and lack of social privileges in the Bantu, in contrast with the diseases found in the well-fed (and often overfed) Europeans, and in the intermediary Cape Coloured group.

In the Bantu group, protein malnutrition is widespread and the factors leading to this are discussed. Cirrhosis and primary cancer of the liver are not uncommon. The suggestion is made that deficiencies of trace elements in the soil may lead to abnormal patterns of aminoacid in the plants grown thereon. Such mineral deficiencies could have an effect on health in areas where the dietary protein is only on the borders of adequacy.

The complex relations between diet, cholesterol metabolism and ischaemic heart disease are discussed. Western privileged culture, operating through lack of exercise, "tension", diet and perhaps other factors, predisposes to ischaemic heart disease. The author is not prepared to make recommendations about dietary fat for populations generally, but a simple and easily

applied modification of European diets is probably advisable for those "at risk" to ischaemic heart disease.

Resistance to infection, stature, longevity and idiosyncrasies and food allergies are also discussed. It is concluded that orthodox medicine has neglected the role of dietetics in the promotion of health and prevention of disease.

[An abstract can, at best, only list the contents of these two stimulating lectures and recommend them as good reading.] *R. Passmore*

FOODSTUFFS

Composition and Bacteriological Control

PIRIE, N. W. **Leaf Protein as Human Food.** *Lancet.* 1959, Nov. 28, 961–2.

A method is described for extracting protein from leaves. The process is relatively simple and the plant inexpensive. Leaf protein has been shown to be of good biological value in experiments on pigs. Trials in England show that it is an acceptable, but not very exciting, food for man. In countries where protein malnutrition is widespread, the introduction of this new food might well have immense effect on health. A great variety of leaves could serve as starting material —wild leaves, waste leaves or special crops. The next step is to try the food out in a country where protein malnutrition is common.

[For this purpose Dr. Pirie is prepared to send batches of 5–50 kgm. leaf protein to anyone "who wishes to undertake a serious study of its acceptability and nutritive value in man." His address is Rothamsted Experimental Station, Harpenden, Hertfordshire, England. It is to be hoped that this offer will find suitable acceptors.] *R. Passmore*

HOBBS, Betty C. **Bacteriology as applied to Imported Foods.** *Sanitarian.* London. 1959, Nov., v. 68, No. 2, 156–62, 167. [10 refs.]

In this paper presented at the Conference of the Association of Public Health Inspectors in September 1959 there is a first-rate expert account of the application of bacteriology to the examination of imported foods. Clear descriptions are given of methods of sampling, current methods of bacteriological testing and of the results of investigation of egg products, meat products and animal feeding stuffs and fertilizers. Some methods for the bacteriological control of foodstuffs are suggested. There are 9 tables of results of the bacteriological examination of non-sterilized canned meat and of egg products, raw meats, feeding stuffs and other samples with stress on salmonella isolations.

In her introduction the author stated "Although the significance of bacterial contamination leading to food spoilage and food poisoning is now well known, the Laws, written and unwritten, with regard to the condemnation of foodstuffs on the grounds of bacterial contamination alone are still far from clear-cut". The important suggestion is made that "there should be international uniformity of technique and bacteriological standards, together with a sharing of knowledge between countries which would help to safeguard the population against exposure to foodstuffs heavily contaminated with food poisoning organisms". *M. E. Delafield*

MOSSEL, D. A. A. & KRUGERS DAGNEAUX, E. L. **Bacteriological Requirements for and Bacteriological Analysis of Precooked ("Instant") Cereals and Similar Foods.** *Antonie van Leeuwenhoek: J. Microbiol. & Serol.* 1959, v. 25, No. 2, 230–36. [20 refs.]

It is suggested that precooked cereals such as baby cereals, precooked wheat, instant rolled oats, corn flakes, puffed rice, instant puddings, dried vegetables and mashed potatoes and soluble cocoa foods should be subjected to the same bacteriological requirements as applied to pasteurized foods, such as milk, ice cream, egg products, cooked sausages and hot snacks, *i.e.*, absence of pathogenic Enterobacteriaceae in 25 gm. food, and of all other Enterobacteriaceae in less than 0·1 gm. food, and a plate count of less than 10^4 per gm. It was thought desirable also to limit the numbers of staphylococci, faecal streptococci, and sulphite-reducing clostridia to less than 10^2 per gm., and of *B. cereus* to less than 10^3 per gm.

85 samples were examined; 94% of the "instant" samples met the requirements but only 63% of the dried vegetable and potatoes reached the standard, and it was concluded that more attention was needed in their preparation and handling. If the tests were limited to a total count and also a count of Enterobacteriaceae 90% of all samples and 83% of samples excluding dehydrated, precooked potatoes and vegetables satisfied the requirements.

The techniques employed are described.

Betty C. Hobbs

FOOD POISONING

BLOSS, J. F. E. **The Assessment of Food Poisoning Hazards.** *Monthly Bull. Ministry of Health & Pub. Health Lab. Service (directed by Med. Res. Council).* 1959, Oct., v. 18, 164–6.

The assessment of food poisoning hazards is an important part of the various food poisoning problems and one at times neglected. The author deals with some of the difficulties and gives an example of a consignment of meat products from Eire in December 1958. It was raw meat minced and pressed into slices and then deep-frozen. The instructions advised as to the cooking required by the consumer. When batches were examined by the port authority concerned the consignment was found to be infected with Salmonella Dublin, coagulase positive staphylococci and *Clostridium welchii*. A discussion at the Ministry of Health between the firms concerned and scientific staff did not lead to absolute destruction or prohibition but to consideration of subsequent cooking times of the foods and the like, and of increased sampling.

The presence of *Cl. welchii* on carcase meat raises problems, for outbreaks are always associated with reheated meat products and lack of attention to good food hygiene practice. Difficulties are discussed.

[In recent years there has been a marked increase in the examination for food poisoning organisms of meat and other foods before they reach the consumer market so that administrative problems like those mentioned by the author are found to increase and it is important to draw attention to the difficulties.]

W. G. Savage

PUBLIC HEALTH LAB. SERVICE. **Food Poisoning in England and Wales, 1958. A Report.** *Monthly Bull. Ministry of Health and Pub. Health Lab. Service (directed by Med. Res. Council).* 1959, Oct., v. 18, 169–78.

This valuable report for 1958 shows generally a similar picture to that of previous years [for 1957 Report, see this *Bulletin*, 1959, v. 34, 364] but with some variations of incidence and of types of the organisms found responsible. The organisms isolated are shown in the following table for general outbreaks, family outbreaks or sporadic cases:

Presumed caus.. agents	General outbreaks	Family outbreaks	Sporadic cases	All incidents
Salm typhi-murium	41	237	3,051	3,329
Other salmonellae	36	91	1,385	1,512
Staphylococci	26	23	76	125
Cl. welchii	69	10	9	88
Sh. sonnei	2	–	–	2
Chemical	–	–	–	–
Not discovered	111	240	1,893	2,244
All agents	285	601	6,414	7,300

Salmonella strains of all types were responsible for 4,841 outbreaks or incidents, apart from those instances where the responsible organism was not ascertained—a constant feature each year. *Cl. welchii* was responsible for a slight increase and caused 24% of the general outbreaks. Until the 2 outbreaks in 1958, *Sh. sonnei* had not been found for a number of years. The figure of 2,244 outbreaks with causes not ascertained is regrettable and this problem has been discussed in earlier reports. [It is largely due to very late reporting and inadequate material available for investigations.]

Table 7 shows, as in previous years, a bewildering variety of salmonella types isolated, but some change in their relative frequency; of the 4,952 strains isolated the most prevalent types were *Salm. typhi-murium*, 3,406 times; Salm. Heidelberg, 308; Salm. Thompson, 132; Salm. Newport, 118; *Salm. enteriditis*, and its varieties, 113; Salm. Saint-Paul, 95; Salm. Derby, 58; *Salm. anatum*, 55; Salm. Bredeney, 50. Of the 79 outbreaks, general and family, due to *Cl. welchii* nearly all were associated with meat, mostly reheated meat. The authors remark "There is so far no sign that caterers are changing cooking methods in order to reduce the risk of food poisoning due to *Cl. welchii*." As regards staphylococcal infections, the general outbreaks fell from 56 in 1957 to 26 in 1958, and family outbreaks from 32 to 23, but sporadic cases increased from 40 to 76.

An interesting part of the report is information as to the foods responsible for food poisoning outbreaks, set out in Table 4. They are divided into Fish 12, meat 140, sweetmeats 11, eggs 6 (duck 3), milk (fresh) 3, cheese 4; vegetables (canned) 4, other foods 1. Another table deals with 117 family and general outbreaks associated with processed or made-up meats. Of them 40 are grouped as reheated meat; 20 meat pies; 7 cold ham or boiled bacon; 16 cold meat; 7 brawn, meat in gelatin, meat roll, potted meat; 5 pressed meat; 8 once cooked stew; 4 sandwiches; 5 sausages; 5 various other meat foods.

Among these outbreaks are a few due to canned meats including outbreaks associated with freshly opened tins. One outbreak of 23 cases came from meat imported from Argentina, 3 outbreaks from Tanganyika imports with 3, 4 and 3 cases respectively, one came from Holland with 4 cases and one from Czechoslovakia with 82 (the only one due to *Cl. welchii*). None were from Britain or the U.S.A.

Dealing with 221 general and family outbreaks, Table 6 gives the places where the contaminated food was bought or eaten. They are canteens 84 (schools 42, works 35, services 6, others 1); restaurants, hotels, cafés etc. 34; hospitals 48; institutions 28; shops 16; private parties 11. [The relative ease of detecting outbreaks in some of these places prevents these figures being fully accepted as representative.] The Report concludes "It becomes clearer each year that food poisoning in England and Wales is largely a matter of salmonellosis. Recent studies have uncovered numerous hitherto unsuspected sources of infection. What is now needed is the application of control measures to the known sources and in particular to egg products. Until control is applied no great change in the incidence of salmonellosis can be expected." *W. G. Savage*

KEMNA, J. & LENK, V. Über eine Typhus-Epidemie nach dem Genuss von infizierten Fleischwaren. (Epidemiologische Untersuchungen bei gleichzeitiger Anwendung der Lysotypie.) [**A Typhoid Epidemic due to the Consumption of Infected Meat Foods. (Epidemiological Investigations with Concurrent Phage Typing)**] *Öffentl. Gesundheitsdienst.* Stuttgart. 1959, v. 21, No. 6, 240–46, 1 fig. [14 refs.]

In the period of late September to early November 1958 a total of 14 cases of typhoid fever were notified to the health authority of Reinickendorf, a town near Berlin. During the same period 13 became known in a district 20 kilometres distant and 2 occurred in 2 other districts. This paper contains a good account of the careful epidemiological investigation that was made and of the results of the phage typing which was carried out. Evidence thus came to light that the factor common to most of the patients was the consumption of meat foods—such as sausages and cooked ham—which were prepared in 1 of the affected districts where they were sold to the public and were also sold in branch establishments in the district 20 kilometres away. It was shown that the meats were probably infected from drainage which because of a defective valve could contaminate the water used in the meat factory. The drainage system received the excreta of a chronic carrier of typhoid organisms of phage type E1a. Organisms isolated from 20 out of the total of 29 patients were found to be of the same type.

M. E. Delafield

ZIKMUND, V., BURIÁN, V., ZIKMUNDOVÁ, V., MARECEK, J. & HÁSEK, V. Rozsáhlá epidemie salmonellózy v krajském městě, vyvolaná salmonellou typhi murium. [**An Extended Epidemic of Salmonellosis in a Regional Town caused by** *Salmonella typhi-murium*] *Československ. Epidemiol., Mikrobiol., Imunol.* Prague. 1959, v. 8, No. 4, 267–72, 1 graph & 1 fig. English summary.

In 1955 an outbreak of *Salmonella typhi-murium* infection occurred among the inhabitants of a town in Czechoslovakia. 105 cases were observed during the first week; the incidence then began to decline, but cases continued to occur for 20 weeks. 110 patients were treated in hospital and the total number of cases was estimated to be 10 times as great. There were 2 deaths. The infection was traced to the consumption of meat from a slaughterhouse in which animals had been suffering from *Salmonella* infection and had been compulsorily slaughtered. The meat had been disinfected by boiling but had been contaminated with *S. typhi-murium* subsequently in the slaughterhouse.

D. J. Bauer

SMITH, Honor V. & SPALDING, J. M. K. **Outbreak of Paralysis in Morocco due to *Ortho*-Cresyl Phosphate Poisoning.** *Lancet.* 1959, Dec. 5, 1019–21, 1 map.

In September 1959 the authors of this paper went to Morocco as "temporary advisers to the World Health Organisation in order to advise the Moroccan health authorities in investigating an outbreak of a paralysing disease of unknown origin", and here give a short account of their personal experience. A full account of the outbreak is in preparation in association with their colleagues in Morocco.

The first cases in the outbreak occurred between August 31 and September 2, 1959. The incidence rose and, between September 18 and 24, 200–300 cases were being reported daily. By October 2 more than 2,000 cases were known and more were to be expected. Both sexes and all ages were liable to the disease but the chief incidence was in adult women, adult men and older children. No cases were seen among the Europeans or the Jews or among the "better-to-do" Moslems. There is a description of the clinical picture of the illness which began with aching pain in the calf, followed by sensory disturbance in the lower limbs which persisted for a day or two and might disappear. Motor weakness of the lower limbs might then set in. Later the muscles of the hand were affected also.

Opinion was at first divided—whether the disease was due to an infection or to poisoning. An interesting account is given of the investigations which provided "overwhelming support" for the toxic theory. It was found that a cooking oil labelled as "olive oil" was being sold in the old Arab town. Analyses of samples of this oil demonstrated the presence of phosphates and cresols. The toxic oil was a "man-made lubricating oil" containing about 33% of vegetable oil and 3% of mixed cresyl phosphates mainly the meta and para compounds, intended originally for turbo-jet engines but which was no longer current for this purpose as its specification was out of date.

There is a discussion of poisoning by ortho-cresyl phosphates. In this described outbreak traces of these ortho-cresyl phosphates were consumed over many days and fortunately the majority of cases were mild although full recovery from the paralysis will take time.

[In a note on this outbreak which follows this paper it is stated that the number of victims of the poisoning is now estimated at 10,000.] *M. E. Delafield*

Mycoses and Mycology

The order of diseases and fungi in this section is:—**General; Dermatophytes; Blastomyces; Histoplasma, Sporotrichum, Phialophora; Candida, Cryptococcus; Actinomyces, Nocardia; Aspergillus; Others.**

Papers on Blastomycosis (South American type), Coccidioidomycosis, Mycetoma and Rhinosporidiosis are abstracted in the TROPICAL DISEASES BULLETIN.

UECKERT, H. Beitrag zur Systematik der Dermatophyten. [**Contribution to the Systematic Classification of Dermatophytes**] *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 176, Nos. 1/2, 102–56, 9 figs. [153 refs.]

This useful review of the dermatophytes for readers of German is based on the extensive literature and on the author's own observations. After a historical introduction and a section on methods, there are descriptions of, and a key to, 4 species of *Microsporum*, 13 of *Trichophyton*, and *Epidermophyton floccosum*, the principal synonyms are given for each species recognized and it may be noted that the author formally proposes, apparently for the first time in line with the International Code of Botanical Nomenclature, the combination *T. mentagrophytes* (Robin) Blanch. var. *persicolor* (Sab.) Ueckert and he also accommodates the dermatophytes in a new tribe Microsporeae of the Moniliales. *G. C. Ainsworth*

GILL, D. **Some Clinical Features of Ringworm in Malaya and Hong Kong.** *J. Roy. Army Med. Corps.* 1959, July, v. 105, No. 3, 120–25, 1 fig.

Body ringworm affecting British troops in Malaya and Hong Kong is found mainly in those newly exposed to the high temperature and humidity; this abnormal susceptibility is short-lived. The lesions are concentrated in areas of clothing tightness and friction. Early complete treatment is essential to avoid a prolonged period of disability but chronic disease is rare.
Peter Abbott

STRAUSS, J. S. **An Epidemic of Human Ringworm due to** *Trichophyton verrucosum*. *New England J. of Med.* 1959, Aug. 13, v. 261, No. 7, 331–3, 2 figs. [13 refs.]

"An epidemic of inflammatory *Trichophyton verrucosum* ringworm in human beings has been described. This epidemic involved at least 16 boys, the largest recorded group of cases occurring in a single farm area, among a group of 50 inmates of a state school for mental defectives. *T. verrucosum* was recovered from the boys and from the involved cows."
[See also this *Bulletin*, 1948, v. 23, 330; 1953, v. 28, 26.]

DONALD, G. F. **A Warning on the Frequency of Endothrix Tinea Capitis among the Aboriginal and Part-Aboriginal Population of South Australia.** *Med. J. Australia.* 1959, Sept. 26, v. 2, No. 13, 435–6.

Writing from Adelaide, the author records the results of a preliminary survey of the incidence of tinea capitis in South Australia and discusses their epidemiological significance.

Between July 1954 and April 1959 white subjects numbering 279 were investigated. Of these, 2 adults and 20 children were found to be suffering from an endothrix type of ringworm. 70 coloured subjects, including 3 aborigines, were also investigated. Of these, all but 1 presented with this type of infection. In this group of 91 infected persons, besides the children who comprised the main body of the patients there were 11 adolescent boys and girls and 5 adult women. This was a very important and significant finding, since the adults were all mothers or grandmothers of infected children under treatment.

The investigation covered 2 groups comprising in each several categories. Thus, in group A there were: Children fostered or adopted by white adults; children living in hostels; aboriginal children in a home; and in group B: children in hospital; and children in an institution. In group A there were 73 (all aboriginal), of whom 32 had an endothrix infection. In group B there were 36 children, of whom 29 were infected.

There appears therefore to be a high rate of infection of the endothrix type of ringworm, not only among whites, but also among half-castes and aborigines. However, the most important step in the control of the spread of this disease has already been taken in so far as trained personnel have been made aware of the situation and are accordingly on the alert for fresh cases. *C. J. La Touche*

MEENAN, F. O. C. & O'CONNELL, L. G. **Preliminary Clinical Observations on Griseofulvin.** *Irish J. Med. Sci.* 1959, Aug., 6th Ser., No. 404, 360–63.

Preliminary clinical impressions after treatment of 24 cases of fungous infections of the skin suggest that Griseofulvin given orally is active against *Microsporon audouini*, *M. canis*, *Trichophyton tonsurans* and *T. schoenleini*. In only one case was there a suggestion of a toxic effect. *Peter Abbott*

FRASER, W. M. & KNOX, J. M. **Amphotericin as a Topical Antifungal Antibiotic. A Clinical Trial.** *Arch. Dermat.* 1959, Oct., v. 80, No. 4, 436–8. [17 refs.]

Owing to favourable reports in the literature concerning the *in vitro* action of the antifungal antibiotic amphotericin against a number of pathogenic fungi and its success or partial success in the treatment of systemic diseases due to fungi, the authors decided to assess its value as a topical drug in various skin mycoses.

154 patients were treated in this manner, and of

these 101 were suffering from one or other of the clinical types of tinea, *viz.*: tinea capitis, tinea cruris, tinea pedis and tinea corporis; of the remainder 25 were affected with pityriasis versicolor and 28 with cutaneous moniliasis.

Results showed that, whereas by and large, the tinea types of lesion failed to respond to topical treatment with this drug, cutaneous moniliasis responded, not only well, but in some cases dramatically, there being only 2 failures which occurred when amphotericin A was used in an ointment containing 2·5 mgm. of neomycin and 0·25 mgm. of gramicidin per gm. in Plastibase. *C. J. La Touche*

SCHERR, G. H. & RIPPON, J. W. **Experimental Histoplasmosis in Cold-Blooded Animals.** *Mycopathologia.* The Hague. 1959, Oct. 15, v. 11, No. 3, 241–9, 2 figs.

These authors describe the results of an interesting series of experiments carried out to ascertain the fate of *Histoplasma capsulatum* in cold-blooded animals. It is well known that, in the tissues, of its normal hosts (mammals) this fungus pursues its parasitic activities in a yeast-like form (yeast phase), whereas during saprophytic existence at normal temperatures it is mycelial in form (mycelial phase).

Frogs and lizards were the animals selected for these experiments. They were inoculated intraperitoneally with suspensions of either the yeast phase or the mycelial phase. The frogs were kept in jars containing a half inch of water; the lizards in damp sphagnum moss. They were divided into groups maintained each at a different temperature, *viz.* 25°, 30° and 37°C. At intervals they were killed and autopsied, and cultures were made from the body cavity fluid and from lesions in the organs on BH 1 blood agar slants at 37°C. and on Sabouraud's agar at 25°C.

Results showed that, irrespective of the type of suspension injected, mycelial or yeast phase, the lesions in frogs maintained at 25° or 30°C. contained only the mycelial phase. The animals which were maintained at 37°C. after being injected all died within 24 hours. In spleen and liver lesions of lizards maintained at 25°C. mycelium was present regardless of whether the yeast or the mycelial phase was injected. At 37°C. yeast was present in these lesions when the yeast phase was injected, but when the mycelial phase was injected mainly mycelium with only occasional yeast was present in these lesions at 9 days, whereas at 13 days mainly yeast was present and only some mycelial, indicating a change from mycelial to yeast phase. It is surmised that a morphological change at 37°C might be due to the presence of certain essential metabolites which are perhaps formed at this temperature, and that by introducing these metabolites it might be possible to effect this change from the mycelial to the yeast phase at 25°C. *C. J. La Touche*

WALKER, Leila & HUPPERT, M., with the technical assistance of A. WOODS. **A Rapid, Reliable Technic for the Identification of** *Candida albicans. Amer. J. Clin. Path.* 1959, June, v. 31, No. 6, 551–8, 6 figs. [17 refs.]

The importance of certain species of *Candida*, especially of *C. albicans*, as pathogens needs no emphasizing. Any claim to improvement in diagnostic technique should therefore command the attention of those who find difficulty in identifying these species.

The corn-meal agar plate and fermentation series have already been widely used with variable success by different workers as also have Weld's eosin-methylene blue technique (EMB) and potato-carrot agar plus bile (PCB). None of these techniques however, can be relied on solely to produce a 100% positive result in the case of *C. albicans* for the reason that many strains of this species exhibit their species characteristics less readily with some techniques than with others. Therefore, to obtain a higher percentage of positive results in the case of *C. albicans* some authors have resorted to the use of several methods concurrently.

These authors are satisfied that the best results are obtainable with a combination of a fermentation series, Weld's EMB method and the corn-meal infusion agar plate method. They devised a "rapid" fermentation test which could be read in 6 hours, and which gave a 98% positive result for *C. albicans*. Results compared very favourably with Weld's EMB and corn-meal agar which gave 88% and 79% positive results respectively. This rapid fermentation test briefly consisted in adding a standardized 1:20 suspension of the yeast to be identified to screwcapped tubes containing sterile sugar solutions plus indicator and incubating at 37°C.

A number of previously identified species and strains of species were tested by a combination of these 3 methods. On EMB and corn-meal agar it was found that some strains of *C. albicans* produced "feathering" but no chlamydospores; some produced both feathering and chlamydospores; and a third group produced chlamydospores but not feathering. In the case of *C. tropicalis*, *C. guilliermondi* and *C. pseudotropicalis* differentiation could be effected by observing their morphological features on corn-meal and their reactions in the rapid fermentation series, and in the case of *C. stellatoidea* production on Weld's EMB agar of a single spiral hypha from each blastospore combined with its reactions in the fermentation series served to distinguish it from other species of *Candida*.

These tests when applied to 97 recently isolated but unidentified species of *Candida* which had formed pseudomycelium on corn-meal agar, gave the following result: *C. albicans* 78; *C. guilliermondi* 7; *C. tropicalis* 7; *C. parakrusei* 2; *C. krusei* 1 and *C. pseudotropicalis* 2. *C. J. La Touche*

NAKAJIMA, S. **Mode of Action of Datemycin on the Growth of** *Candida albicans.* **I. Agglutination Reaction of** *Candida albicans* **observed in the Presence of Datemycin.** *Tohoku J. Exper. Med.* 1959, July 25, v. 70, No. 2, 103–13, 1 fig.

"In the presence of an antibiotic, datemycin, the agglutination phenomenon of *C. albicans* was first recognized. Further study revealed that the agglutination reaction was restricted to only susceptible strains, and these cells have to be in vegetative state

but not killed. The reaction was evident only in room or blood temperature but not in the cold. The optimum pH was in the vicinities of 7.0. Sugars which are utilized by the tested organisms had a stimulating effect on the agglutination. Further microscopic examination revealed that the mycelium formation of the tested cells was the real mode of this agglutination.

"In a slide cell culture system, the fact that the lower concentration of datemycin stimulates the mycelium formation of Candida was quite evident. Both agglutinative and inhibitory activities of datemycin were partly reversed by the addition of cysteine."

SHIELDS, L. H. **Disseminated Cryptococcosis producing a Sarcoid Type Reaction. The Report of a Case treated with Amphotericin B.** *Arch. Intern. Med.* 1959, Nov., v. 104, No. 5, 763–70, 9 figs.

HOLM, P. & KWAPINSKI, J. B. **Studies on the Detection of *Actinomyces* Antibodies in Human Sera by using Pure Antigenic Fractions of *Actinomyces israeli*.** *Acta. Path. et Microb. Scandinavica.* 1959, v. 45, No. 1, 107–12.

The aim of these investigations was to detect and examine, by using isolated chemical fractions of *Actinomyces israeli*, anti-actinomyces antibodies in sera derived from patients suffering from actinomycosis.

4 representative antigenic fractions (3 nucleoproteins and 1 polysaccharide) derived from 4 strains of *Actinomyces israeli* which were isolated from pus obtained from human actinomycosis were selected for the investigation. A table shows the main properties of each antigenic fraction, *viz.* general chemical designation; percentage content of phosphorus, albumin and sugar; and serological activity in rabbit antisera.

81 human sera were used. Of these, 14 were from patients suffering from the cervico-facial form of actinomycosis, 22 from tuberculous patients, 19 from patients suffering from streptococcal infections, 6 from seropositive syphilis and 20 from clinically healthy blood donors. These sera were inactivated and preserved in the frozen state until required.

Both haemagglutination and complement-fixation tests were used, and details of procedure followed in these tests are described by the authors. The quantitative results obtained thereby are recorded by them in tabular form.

Antibodies in 12 out of 16 samples of serum from patients suffering from actinomycosis were found to react with isolated fractions of *Actinomyces israeli*.

There was some evidence of serological cross-reactions between anti-*Actinomyces* and anti-*Mycobacterium* antibodies, but to a far less extent between anti-*Actinomyces* and anti-*Streptococcus* antibodies.

C. J. La Touche

GODFREY, L. B. **Aspergillosis of the Lung.** *Irish J. Med. Sci.* 1957, Sept., 6th Ser., No. 381, 407–11, 3 figs.

3 cases of pulmonary mycetoma are presented. The characteristic X-ray appearance of an "ovoid cavity with relatively thin walls containing a mass sharply demarcated from the surrounding walls with an intervening free crescentic area" is stressed as is the frequency of the disease being misdiagnosed as tuberculosis.

Peter Abbott

NAJI, A. F. **Bronchopulmonary Aspergillosis. Report of Two New Cases, Review of Literature, and Suggestion for Classification.** *Arch. Pathology.* 1959, Sept., v. 68, No. 3, 282–91, 8 figs. [32 refs.]

2 cases of bronchopulmonary aspergillosis are presented. The first patient had a single fungus mass in a cavity in the right apex and this was successfully removed by right upper lobectomy. The second, an elderly woman who died with agranulocytosis, was shown at autopsy to have multiple lesions throughout both lungs made up of confluent areas of bronchopneumonic consolidation in which were seen numerous septate, branching filaments. *Aspergillus flavus* was identified by culture.

The author reviews the literature on bronchopulmonary aspergillosis and emphasizes that these cases illustrate the 2 main types; (1) intracavitary fungus mass or intracavitary aspergilloma, and (2) bronchopneumonic aspergillosis. He gives tables showing the main features of 20 cases of the first type and 17 of the second type described in previous reports.

Peter Abbott

EVOLCEANU, R. & ALTERAS, I. Considérations à propos des caractères mycologiques et pathogéniques du *Keratinomyces ajelloi* Vanbreuseghem 1952, saprophyte du sol. [**Remarks on the Mycological Features and Pathogenicity of the Soil Saprophyte, *Keratinomyces ajelloi* Vanbreuseghem, 1952**] *Mycopathologia.* The Hague. 1959, Oct. 15, v. 11, No. 3, 196–204, 6 figs. [13 refs.]

After briefly reviewing the relevant literature the authors describe the macroscopic and microscopic features of 2 strains of *Keratinomyces ajelloi* isolated by them from soil in Bucharest. They then proceed to describe the results which they obtained from experimental inoculation of laboratory animals and Man with these strains. Finally, they record briefly the results of skin tests made with an extract of these strains.

On Sabouraud test agar colonies grew quickly, reaching up to 8 cm. in diameter. The surface growth resembled fine plaster and was ochraceous in colour. A mahogany-red diffusible pigment was produced by this growth.

In Van Tieghem hanging-drop cultures for which Sabouraud agar was used the most characteristic feature was the abundance of macroconidia in which the authors see a resemblance to those of Epidermophyton. No microconidia were observed. A few tightly coiled spirals were present in cultures on the standard media as well as chains of chlamydospores.

The authors succeeded with some difficulty in inducing a single lesion only in one human subject and likewise in a mouse and a guineapig with one of these

strains. In the human subject the lesion was observable at 5 days after inoculation as an erythematous patch, and during the next few days pin-head vesicopustules developed within it. These contained fairly stout hyphae. This lesion disappeared spontaneously 7 or 8 days later. In the guineapig and in the mouse an erythematous lesion appeared 7 to 8 days after inoculation. It was covered with fine scales which, on microscopical examination revealed stout hyphae. In no case were the hairs invaded. Spontaneous resolution took place 7 or 8 days later. Retroculture from these lesions reproduced all the features observed in the strain used as the inoculum.

Extracts were prepared from cultures 5 to 6 weeks old of these strains on Sabouraud broth. After the mycelium had been washed in running water it was dehydrated in acetone and powdered. This material was then suspended in distilled water (1:9 parts distilled water) kept at 80°C. for 1 hour, and centrifuged. The supernatant fluid was then dialysed in running water for 2 days and again in distilled water and finally dried. This extract, when diluted 1:500 and injected intradermally into patients suffering from inflammatory mycotic skin infections, elicited from them a positive reaction, whereas no reaction was obtained from the controls.

The authors conclude from their observations that *K. ajelloi* should be included amongst the Dermatophytes and propose that, on account of its lack of microconidia (aleuries) and its possession of spirals (vrilles) it should be regarded as a primitive species of Epidermophyton, namely, *Epidermophyton terrigenum*.

C. J. La Touche

Communicable Diseases

The order in this section is:—**Tuberculosis; Venereal Diseases; General Infective Diseases; Other Communicable Diseases.**

For the bacteriology of these diseases see the section on Bacteriology below. For diseases conventionally regarded as tropical, readers are referred to the TROPICAL DISEASES BULLETIN.

TUBERCULOSIS

ROE, J. T. N. **Tuberculosis in Indian Immigrants.** *Tubercle.* 1959, Oct., v. 40, No. 5, 387–8.

The matter of tuberculosis in immigrants to Great Britain continues to receive attention, and in the face of declining morbidity in the native population of England and Wales it becomes increasingly important.

Between 1954 and 1958 Southall (population 53,000), an industrial borough near London, received some 1,050 Indian immigrants. During the same period there was a steady increase in the tuberculosis notification rate in Indians in the borough so that the health authorities investigated the situation.

The case records of 35 patients were available for examination (32 males and 3 females); 5 patients had left the district. 10 patients had primary pulmonary lesions with manifest enlargement of lymph nodes and 4 had pleural effusions. 1 developed miliary disease without an evident primary complex, and 2 with primary complexes also developed miliary disease.

15 patients had what appeared to be post-primary pulmonary tuberculosis; there was cavitation in 5 of these. The disease was extra-pulmonary in 5 (cervical adenitis 3, kidney 1 and knee 1).

In spite of a very wide investigation of home contacts no source of infection was discovered. The husband of one of the women had a primary lesion which, it was thought, he had acquired from his wife.

Most of the patients were Sikhs; the 3 women were all from villages and had been associated with farming. 23 men were from village or country, 18 being farmers. Only 6 men were from town or city.

Of the 35 patients 21 were thought to have acquired the disease in England and 2 possibly to have acquired it after arrival; 12 persons probably had the disease on entry—10 with pulmonary and 2 with non-pulmonary tuberculosis.

These findings and conclusions are contrasted with those of SPRINGETT *et al.* [this *Bulletin*, 1959, v. 34, 33] who concluded that among Indians and Pakistanis in Birmingham the disease was predominantly of a chronic post-primary type and that most of the immigrants were already tuberculous on arrival in England.

Roe's findings are supported by the added finding that of 44 other Indians who were tuberculin tested from September 1958 (Mantoux 10 TU of OT) 29 failed to react. He advises that all Indians be X-rayed as soon as possible after arrival and tuberculin tested; BCG vaccination should be offered to non-reactors. Efforts should also be made to improve their living standards. [See also this *Bulletin*, 1958, v. 33, 566].

H. G. Calwell

MERRETT, J. D. **Social Class Distribution of Respiratory Tuberculosis Mortality in Northern Ireland, 1949–53.** *Brit. J. Social & Preventive Med.* 1959, July, v. 13, No. 3, 156–61, 1 fig.

During the period 1949–53, 1,254 deaths of males aged 15 years and over in Northern Ireland were attributed to respiratory tuberculosis.

There were 20 deaths in Social Class I (expected deaths 31·62), 156 in Class II (expected 316·44), 502 in Class III (expected 447·85), 117 in Class IV (expected 230·85) and 403 in Class V (expected 198·6). The remaining 56 deaths were of "unoccupied" persons

(4·5% of the total); the number of expected deaths was 28·64.

When standardized mortality ratios were compared it was seen that mortality in Classes I and II combined in England and Wales was nearer to the average mortality of those countries than was mortality in these classes in Northern Ireland to the average of that region. In Class III the mortality in Northern Ireland was slightly greater than in England and Wales at all ages under 65 years.

For Social Class IV the Northern Ireland rates in each group were found to be lower than those for England and Wales. The more favourable experience in Northern Ireland appeared to be due to the relatively high proportion of agricultural workers in the Class (50% compared with 26% in England and Wales). In both countries the standardized mortality ratios are much lower than those for the remaining partly-skilled workers in Class IV.

For Social Class V the rates suggest that males in Northern Ireland have a much higher death rate from respiratory tuberculosis than those in England and Wales. In this Class in Northern Ireland 24·4% of all males were classified as labourers compared with 21·9% in England and Wales. However in Northern Ireland it was found that about 88% of all deaths from respiratory tuberculosis in Class V were of labourers compared with 39% in England and Wales.

For those classified as labourers age specific death rates in Northern Ireland are 3–12 times larger than the corresponding rates in England and Wales while in the other occupational groups of Class V the rates in Northern Ireland are consistently lower than in England and Wales.

The difference may be due to a social factor such as the persistently high level of unemployment in Northern Ireland or to inexact classification of jobs at death registration. It is imposible to decide which of these explanations is the more likely. Comparison of Classes IV and V combined shows that up to age 65 the rates in Northern Ireland are greater than those in England and Wales but thereafter the reverse is true.

H. G. Calwell

LOTTE, A. & ROUILLON, A. Rapport sur le fonctionnement des dispensaires antituberculeux français en 1957. **[Report of the Tuberculosis Dispensaries in France in 1957]** *Bull. Inst. Nat. Hyg.* Paris, 1959, Sept.–Oct., v. 14, No. 5, 861–916, 13 figs.

This report does not present the whole picture of tuberculosis in France as only a part of the tuberculosis service is provided by the dispensaries dealt with and in any case notification of the disease is neither systematic nor compulsory.

According to the 1956 report [this *Bulletin*, 1958, v. 33, 1116] the total number of tuberculosis dispensaries in France was 954; in 1957 there was 950 of which only 947 submitted returns. The average population of a dispensary area was 45,572.

The total number of consultations was 1,696,437, an increase of 1%, and the number of patients attending for the first time was 537,976, a decrease of 4%. These totals took no account of persons who attended for X-ray examination in mass groups who numbered 1,317,085, an increase of 4%.

The number of tuberculin tests was 368,913; almost 500,000 persons were vaccinated with BCG but many vaccinations were not reported to the dispensaries and the total given is short of the number for whom compulsory vaccination is prescribed by law.

Admissions to hospitals numbered 16,788, to sanatoria 25,386 and to preventoria 16,722; all showed a slight decrease. There were also fewer families to supervise as contacts. The number of pneumothorax refills fell from 90,103 to 46,501. Only one-half of the total number of tuberculosis deaths in France were notified by the dispensaries.

There were 43,894 new cases of tuberculosis (100/100,000); 27,932 (132/100,000) of these patients were males and 15,962 (71/100,000) females. The incidence of the disease varied greatly in different areas. There was a decline of 11·5% compared with 1956. This is regarded as a true decline.

New cases were analysed according to age, sex and clinical form of the disease. Primary tuberculosis manifested radiologically was commonest at school age, and post-primary pulmonary tuberculosis increased rapidly after the age of 15 years. Pleurisy and extrapulmonary disease was a little commoner under the age of 30 than above it. Post-primary pulmonary tuberculosis was commoner in males than females after the age of 25.

The report contains many graphs and nomograms and 22 tables giving full details for each Department of France. These would be invaluable for any student of the epidemiology of tuberculosis in that country.

H. G. Calwell

FREUDENBERG, K. Statistischer Beitrag zum Stand des Tuberkuloseproblems. **[Statistical Analysis of the Incidence of Tuberculosis]** *Öffentl. Gesundheitsdienst.* Stuttgart. 1959, v. 21, No. 6, 226–39.

In this paper there is a statistical analysis of the data of tuberculosis incidence in the Federal Republic of Germany. These data are set out by age and sex in numerical tables. There is also a table in which tuberculosis mortality rates by age and sex in Prussia in 1908 and the Federal Republic in 1956 are compared.

In the last 50 years mortality from tuberculosis has been reduced to a very small fraction of the original figure in the young age-groups but the reduction in the higher age-groups has been much less evident. This phenomenon is also to be seen in statistical study of the morbidity figures as for example that in a comparison of tuberculosis incidence in Hesse in 1952 and in 1956 there occurred a 19% reduction of aggregate incidence for all age-groups but the reduction was most marked in the lowest age-groups, less marked in the next higher age-groups with an actual increase of incidence in age-groups of 55 years and over. There is a full discussion of these findings which it is thought may be explained by the fact that in past years in schemes for the ascertainment of tuberculosis infection attention was focused on the lower age-groups but that with the development of these schemes diagnosis in the older age-groups has become more complete.

M. E. Delafield

YAMAGUCHI, M., OKA, H., KUMABE, H. & YOSANO, H. **Survey of Tuberculosis Prevalence in Japan, 1954: Trends in Tuberculosis from 1953 to 1954.** *Bull. World Health Organization.* Geneva. 1959, v. 21, No. 2, 145–59, 9 figs.

In 1953 the Ministry of Health and Welfare conducted a survey of the prevalence of tuberculosis in Japan (population about 91,000,000). The survey was based on sampling in 210 areas, and from the results it was estimated that there were 2,292,000 persons (3·4% of the total population) in need of treatment for tuberculosis.

In 1954 a second survey was conducted in 70 of the areas with the object of assessing the trend as shown by tuberculin reactor rates, incidence of morbidity and type and extent of disease. Details of the sampling methods, the organization and execution of the surveys and the diagnostic standards applied are given.

Of the 14,793 persons tested with tuberculin both in 1953 and 1954, 6,864 (46·4%) had been vaccinated with BCG in the past and 2,512 of the 5,819 non- or doubtful reactors (43·2%) were vaccinated in the interval. Excluding these the reactor rate was much higher in 1954 (77·8%) than in 1953 (68·1%), and in spite of uncertainty connected with the test itself the authors feel that heavy infection took place during the year. The conversion rate was 31·7% and the reversion rate 2·1%.

The number of new cases of pulmonary tuberculosis found in persons who in 1953 had healed lesions or no evidence of the disease was 54 (0·4%). Extra-pulmonary tuberculosis was found in 13 persons (0·1%). The types of pulmonary disease were: primary 16 (29·6%), pleurisy 3 (5·6%) and infiltrative or mixed 35 (64·8%). Definite cavitation was not seen. The disease was far advanced in 4 (7·4%), moderately advanced in 4 and minimal in 46 (85·2%).

Of the 921 persons who were found to have pulmonary tuberculosis in 1953, 30·3% had improved, 15·8% were worse, 53·3% showed no change and 0·7% had died. No significant difference was found by sex but when the results were analysed by age it was seen that children had fared best and that the rate of improvement decreased with age. The prognosis was best with primary disease.

Of 515 persons for whom treatment had been indicated in 1953, 187 (36·3%) had received some during the year, most of them chemotherapy. The highest acceptance rate was in the age-group 15–29 years. Only 17·4% of those for whom hospital treatment was advisable were in fact admitted. In 1954, 68 persons (26·5% of those treated) were admitted to hospital.

Of those patients who required treatment in 1953 81·5% still required it in 1954 and 16·9% required rest or observation. Of those who were found to require observation in 1953 84·5% still required it in 1954, 12·8% now required medical treatment and 1·6% had recovered. *H. G. Calwell*

BRITISH TUBERCULOSIS ASS. **A Single Tuberculin Test for Epidemiological Use: a Comparison of the Mantoux and Heaf Tests. A Report to the Research Committee by their Tuberculin Sub-Committee** [CROFTON, J., Chairman]. *Tubercle.* 1959, Oct., v. 40, No. 5, 317–35, 5 figs. [22 refs.]

In a previous report of the British Tuberculosis Association [this *Bulletin*, 1958, v. 33, 831] the results given indicated that the Mantoux (intracutaneous) and Heaf (multiple puncture) tuberculin tests should be investigated further and compared with the object of finding the best single test for epidemiological use.

The further work now described was carried out at 25 centres in Great Britain. The co-operating physicians were asked to test any convenient population groups excluding persons known to have or to have had tuberculosis or to have been tested within the previous 6 months. Persons vaccinated with BCG were eligible but being few in numbers they were excluded from the analysis.

The total number tested on both arms was 3,640. PPD (2 mgm./ml.) was used for the Heaf test and dilutions containing 5 and 100 TU for the Mantoux test. Each subject received 1 test on either forearm, and the results were read 3, 7 and 10 days later. The criterion of a positive Mantoux reaction was induration of 6 mm. or more and Heaf's criteria were used for the multiple puncture test.

78·5% reacted to the Mantoux test (45·5% to 5 TU at 3 days, 45·6% at 7 days and 24·1% at 10 days). The mean diameter of induration decreased from 10·9 mm. to 9·5 mm. between the 3rd and 7th day. Between the 7th and 10th day only 1·3% changed from negative to positive whereas 22·9% changed from positive to negative.

For the 100 and 5 TU tests combined the percentage positive at 3 days increased from 19·5% at 0–14 years to 92·9% at 45–64 years and 79·9% over 65 years. The highest proportion positive to 5 TU was at 25–44 years. In all age groups the proportion positive at 10 days was much lower than at 3 or 7 days.

The relationship with age noted above was also found to hold for each sex separately but reactor rates throughout were higher in males than in females. The observers placed most confidence in their reading of the 5 TU test at 3 days.

Among 1,656 persons reacting to the 5 TU test severe reactions were noted in 25 (1·5%); in 990 reactors to 100 TU 69 (7·0%) gave a severe reaction.

With the Heaf test the reactor rate was 74·3% on the 3rd day, 78·0% on the 7th day and 78·3% on the 10th day. 4·7% became positive between the 3rd and 7th day and 1·0% became negative. Between the 7th and 10th day 3·0% became positive and 2·6% negative.

The reactor rate increased from about 20% at 0–14 years to 90% at 45–64 years and declined to 80% in the oldest age-group. For all ages combined 81·4% of males reacted at 7 days compared with 74·2% of females. The differences between the sexes was small at 0–14 and negligible at 15–24 years.

Doubtful reactions were seen in 4·2% at 3 days and in 4·1% at 10 days. The proportion of doubtful reactions was greater at 25–64 years than in the other

age-groups. Severe reactions were seen in 264 (9·3%) of 2,824 reactors.

The results are compared. The Heaf test gave more positive results than the Mantoux test with 5 TU and a very similar proportion of positive results to the 100 TU test. It was easier to perform and to read than the Mantoux test; the tuberculin used was more stable and a longer interval between testing and reading was possible. Although severe reactions are commoner with the Heaf than with the 5 TU test none of the observers in this trial regarded them as sufficiently troublesome to condemn the method.

The conclusion is that for epidemiological use the Heaf test is to be recommended as an efficient one-stage test. *H. G. Calwell*

POLLOCK, T. M., SUTHERLAND, I. & HART, P. D'A., with the assistance of Anne HARRIES, Joan LEACH, Katherine MERCER, Christine MILLER & Barbara SIMONDS. **The Specificity of the Tuberculin Reaction in Man in Great Britain.** *Tubercle.* 1959, Oct., v. 40, No. 5, 336–54, 2 figs. [30 refs.]

The main purpose of this enquiry was to study the possibility that positive reactions to a high dose of tuberculin by persons with negative reactions to a low dose are due to an agent other than the tubercle bacillus.

Between 1954 and 1956 recruits to the Royal Air Force from all parts of the United Kingdom were invited to participate in the trial. They were asked about previous contact with a person requiring treatment for pulmonary tuberculosis and, according to the history, were classified as having had close, moderate, slight or no contact.

The number of volunteers available for final analysis was 7,646 of whom 1,211 had been in close contact, 922 in moderate, 401 in slight and 5,112 in no contact with tuberculosis.

In the first phase of 42 weeks all received 0·3, 1 and 3 TU of OT intracutaneously in the left forearm, and if there was induration of less than 5 mm. diameter to 3 TU they were given 10, 30 and 100 TU in the right forearm. In the 2nd phase of 15 weeks the same procedure was followed but the criterion of a positive reaction was put at 8 mm. or more of induration. During the final 20 weeks all were tested on the left forearm with 1 and 3 TU; if there was less than 8 mm. of induration to 3 TU the participant was tested on the left forearm with 10 TU and on the right forearm with 10, 30, 100 and 1,000 TU.

The percentages of reactors to 3 TU were 81·5, 65·0 and 56·1 in those with close, moderate and no contact respectively and to 100 TU (in those negative to 3 TU) 27·2, 30·2 and 30·0 respectively.

The percentages reacting to 1 TU were 80·0, 62·1 and 53·0 respectively, to 10 TU (in those not reacting to 1 TU) 15·7, 15·4 and 14·5 respectively and to 3 TU 9·1, 9·4 and 8·2. There was lack of association with degree of contact in the last 2 series.

The cumulative proportions found to react to increasing doses of tuberculin from 0·3 to 1,000 TU according to degree of contact were examined. In the close contact group they were 77·8–88·3%, in the moderate contact group 58·8–80·5%, in slight contact 50–59·5% and in the no-contact group 49·1–76·6%.

The percentages of those positive to increasing doses of tuberculin among those negative to the next lower dose were examined in relation to the degree of contact. There was some association between degree of contact and reactor rates with 1 TU among those negative to 0·3 TU. Above 1 TU, however, no obvious relationship was found.

The distribution of induration with increasing doses was examined. With doses of 0·3, 1 and 3 TU there was a large proportion of reactions with no induration, much smaller proportions with induration of 2–5 mm. and a secondary peak with induration of large diameter.

With doses of 1–1,000 TU the results in those who had negative reactions to the next lower dose showed a large proportion with no (or only 1 mm.) induration and progressively smaller proportions with induration of large diameter. There was no secondary peak as with the smaller doses. This distribution is characteristic of a group from which all who are likely to show large reactions to the particular dose of tuberculin have been excluded. There was no essential change in the type of distribution with increasing dose of tuberculin.

The average diameter of induration increased with the dose of tuberculin and in less amount with the degree of contact with tuberculosis.

As stated the participants came from all parts of the United Kingdom. It was found that there were geographical differences in the reactor rates to 3 TU in each contact group, the rate being highest in those from Scotland or Northern Ireland and least for those from southern England; no regional differences were seen in the proportions positive to 100 TU among those negative to 3 TU.

The conclusion reached was that the non-specificity to tuberculin which PALMER and his colleagues claim to have demonstrated in the United States and elsewhere [this *Bulletin*, 1954, v. 29, 253; 1955, v. 30, 214; also EDWARDS *et al.*, *ibid.*, 1959, v. 34, 208, and 1960, v. 35, 132] has not been demonstrated in the United Kingdom by the present enquiry. "... weak degrees of tuberculin sensitivity in man in Britain appear to be explicable on the basis of tuberculous infection." *H. G. Calwell*

PALMER, Carroll E., EDWARDS, Lydia B., HOPWOOD, Louise & EDWARDS, Phyllis Q. **Experimental and Epidemiologic Basis for the Interpretation of Tuberculin Sensitivity.** *J. Pediatrics.* St. Louis, 1959, Oct., v. 55, No. 4, 413–29, 9 figs. [12 refs.]

The present paper reports further work on the interpretation of tuberculin sensitivity based on the results of experimental work in guineapigs.

There were 424 guineapigs in the experiments of which 152 had been infected with the Battey organism [this *Bulletin*, 1957, v. 32, 659], 147 with virulent tubercle bacilli, 87 with the Battey organism and later with virulent tubercle bacilli and 38 had been left uninfected as controls.

Animals from the different groups were housed together and all were tested intracutaneously with both PPD-S and PPD-B [Battey] the dose being 0·0005 mgm.

In those animals infected only with virulent bacilli the reactions to PPD-S were concentrated between 4 and 16 mm. (average 10·4 mm.). Most also reacted to PPD-B but in smaller degree (average 6·2 mm.).

In animals infected only with the Battey organism reactions to PPD-B were concentrated between 4 and 12 mm. (average 7·5 mm.). Reactions to PPD-S were small (average 3·1 mm.).

In guineapigs infected only with virulent tubercle bacilli or the Battey organism the reaction to the homologous antigen was with few exceptions larger than that to the heterologous antigen. The results suggest that if the larger reaction of the pair had been taken to indicate the identity of the source of infection the diagnosis would have been correct in 90% of the 299 animals, wrong in 4% and doubtful in 6%.

In the guineapigs infected with the Battey bacillus and later superinfected with virulent tubercle bacilli, reactions to PPD-B were essentially the same as those in the group infected only with the Battey bacillus (average 6·9 mm.). Reactions to PPD-S were essentially the same as those in animals infected only with H37Rv (average 11·2 mm.).

These results suggest that in guineapigs infection with the Battey bacillus does not affect the degree of sensitivity to PPD-S produced by a later virulent superinfection and that a later virulent superinfection does not affect sensitivity to PPD-B due to a previous infection with the Battey bacillus. Thus even if a Battey infection has preceded a virulent infection it is still possible by comparing the sizes of the reactions to either tuberculin to identify most of the animals that have had a virulent infection. In general, however, the converse is not true, which is of less importance.

Frequency histograms of the sizes of reactions to PPD-S for theoretical populations made up of various proportions of guineapigs infected with virulent and Battey bacilli and uninfected were studied.

In a population of uninfected and tuberculous infected animals the pattern with PPD-S was bimodal with 1 modal group of the uninfected and the other of the infected centred at 10–11 mm. There was an overlap in which both infected and uninfected had reactions of equal size. By selecting a criterion which will provide the most efficient separation of the 2 groups it is possible to use the tuberculin test to divide such a population with a high degree of precision.

In a population consisting of uninfected, tuberculous infected and Battey infected guineapigs the pattern of sensitivity varies according to the proportion of each group in the whole. When the proportion of tuberculous infected is high and that of Battey infected low the histogram will show a peak at 10–11 mm. When the proportion of tuberculous infected is low the peak is shifted to 4–5 mm. Identification of the tuberculous infected in such a population cannot be done very satisfactorily with the tuberculin test alone.

The above experimental findings are compared with the observed patterns in human populations already reported [this *Bulletin*, 1957, v. 32, 131]. Some were of patients in tuberculous hospitals and others of general and special population groups in the United States and elsewhere. Cross-reactions to tuberculin were found to be very prevalent in some areas and not in others. The frequency of cross-reactions was found to vary with the prevalence of sensitivity to PPD-B but whether or not the Battey organism or some other related organism is chiefly responsible is not known. Simultaneous testing with PPD-S and other PPD antigens could help to answer the question. [See also this *Bulletin*, 1954, v. 29, 253; 1959, v. 34, 208; 1960, v. 35, 132.] *H. G. Calwell*

See also p. 236, Kröber. [**Occupational Tuberculosis of the Skin.**]

Steel, S. J. & Bentley, F. J. **Advances in Treatment of Chronic Pulmonary Tuberculosis in Children.** *Tubercle.* 1959, Oct., v. 40, No. 5, 377–83.

This paper compares the results of treatment in 67 children who came under treatment at the High Wood Hospital, Brentwood, Essex, in 1947–8 with the results obtained on 71 children admitted in 1954. On the whole the numbers of advanced cases were greater in the first group, otherwise they seem comparable. The first group were treated by rest and collapse therapy. Of these, 36 remained well; 15 had died; 14 had non-fatal relapses; and 2 were steadily deteriorating.

This assessment was based on the condition at the end of 3 years, so that it was comparable with the second group. In the second group treated with antimicrobial drugs and collapse therapy 69 were well at the end of 3 years, and only 2 had had relapses, both of whom were successfully retreated. Surgery was performed in the 1954 group in 8 patients for persistent cavities and dense residual lesions. Pneumothorax has now been given up, and very little surgery is performed. *F. H. Young*

Boyd, D. H. A., Stewart, Sheila M., Somner, A. R., Crofton, J. W. & Rees, R. J. W. **Macrocyclon in the Treatment of Pulmonary Tuberculosis.** *Tubercle.* 1959, Oct., v. 40, No. 5, 369–76. [20 refs.]

Up to now the effective drug treatment of tuberculosis has been confined to the use of agents which are bacteriostatic or bactericidal to the tubercle bacillus *in vitro*. Macrocyclon, however, is used with the idea of modifying the host response. In 1951, Cornforth *et al.* reported the suppressive effect on moderately acute tuberculous infection in mice of an arylalkyl polyether of phenol [this *Bulletin*, 1952, v. 27, 50; 1955, v. 30, 752]. Macrocyclon is one of the most active and least toxic forms of this compound.

After a trial of toxicity in mice and rhesus monkeys this compound was tried on 10 advanced cases. The usual assessments were made clinically, radiologically and pathologically before the assessment of treatment. No other chemotherapy was used during the trial. Macrocyclon as a 12·5% solution in 0·45% saline was given once each week intravenously by increasing doses starting with 4 cc. until a dose of 50 mgm. per kgm. body weight was reached, and subsequently this dose was doubled. No immediate side effects were encountered, the only change being a rise in the serum

cholesterol level. In spite of the satisfactory results in experimental animals no appreciably favourable result was obtained on the 10 patients. The authors think that this was due to the fact that in man with pulmonary tuberculosis the tubercle bacilli are not normally in the monocytes. *F. H. Young*

LAVERS, K. W. & ROBERTS, J. C. **The Use of Prednisone in Primary Tuberculosis in Children.** *Tubercle.* 1959, June, v. 40, No. 3, 173–6.

The use of antimicrobial drugs in primary tuberculosis in children has in most hands produced disappointing results. The addition of steroid therapy has been tried in a comparatively small number of cases but has not produced in ordinary cases any dramatic effect, though in those severely ill more encouraging effects have been reported. This paper deals with 10 children in whom about 3 months or more treatment with antimicrobial drugs had produced little effect.

2·5 mgm. prednisone was given to the younger and 5 mgm. to the older children daily with continuation of the antimicrobial drugs in the usual doses. In 8 patients improvement took place, in 7 the improvement was considered to be associated with the prednisone. No significant side effects were noted. A table with sketches of the X-rays before and after treatment illustrates the effects very clearly. There is a suggestion that any improvement shown will be completed at the end of 3 months' treatment. *F. H. Young*

KENDIG, E. L., Jr. & HUDGENS, R. O. **The Effect of Rubeola on Tuberculosis under Antimicrobial Therapy. I. Primary Pulmonary Tuberculosis treated with Isoniazid.** *Pediatrics.* Springfield, Ill. 1959, Oct., v. 24, No. 4, 616–18. [15 refs.] **II. Tuberculous Meningitis treated with Isoniazid, Streptomycin and Para-Aminosalicylic Acid.** *Ibid.*, 619–22.

I. It has been held by many authorities that the incidence of measles in patients suffering from pulmonary tuberculosis causes a worsening of the latter. This article reports an examination of the effect of measles on children with active primary tuberculosis already under treatment with isoniazid. 12 children had typical primary tuberculosis demonstrable on X-rays by enlargement of the hilar lymph nodes and in 2 cases with infiltration into the base of the right lung. Gastric cultures were positive in 2; all patients had positive tuberculin reactions. 8 of the children received isoniazid in doses of 10 mgm./kgm. of body weight per day, and 4 received 5 mgm./kgm.; in none of the children who developed measles was there any instance of worsening of the tuberculous process. The article goes on to consider the evidence that measles does exert a deleterious effect on tuberculosis.

II. In the previous article the evidence that tuberculosis is unfavourably affected by measles was examined. This paper reports the effects of measles on 5 children suffering from tuberculous meningitis and under treatment with isoniazid, streptomycin and PAS. Full case reports are given, including cerebrospinal fluid pathological findings, just before and after the start of measles. In none of the 5 children did there appear to be any deleterious effect as a result of the measles. The authors quote various articles which suggest that measles has an unfavourable effect on tuberculous meningitis, but in all these treatment by streptomycin alone was in progress.

F. H. Young

DONOHOE, R. F., DUKE, C. J., KATZ, S. & ROMANSKY, M. J. **A Clinical Evaluation of Thiocarbanidin and Isoniazid in the Treatment of Pulmonary Tuberculosis.** *Amer. Rev. Respiratory Dis.* 1959, Oct., v. 80, No. 4, Pt. 1, 590–93, 1 fig. [Refs. in footnotes.]

Thiocarbanidin has been shown to have definite antituberculous activity in animals [this *Bulletin*, 1958, v. 33, 696]. It has been suggested that it might prove an adequate substitute for PAS. 41 patients with active pulmonary tuberculosis were treated with thiocarbanidin in a dosage of 2 gm. daily given in 4 divided doses. 40 of these received isoniazid in addition, and 1 dihydrostreptomycin pantothenate. No gross toxicity or intolerance was encountered. 25 patients were considered to have a favourable clinical response; sputum conversion occurred in 20 of these, but in 2 of the 5 patients in whom reversal of infectiousness did not occur organisms resistant to isoniazid appeared. In general, radiologically, the results were poor and inferior to those encountered with isoniazid + PAS, or other standard regimes. Of the 21 patients in whom bacteriological conversion did not occur 15 (71%) excreted tubercle bacilli resistant to the companion drug; in all, the bacilli before treatment were susceptible to the companion drug.

As a result of this study the authors consider that thiocarbanidin in its present form would be an ineffective antituberculous agent when employed in a dosage of 2 gm. daily. *F. H. Young*

LEITES, Vera. **Cycloserine-Isoniazid in the Ambulatory Treatment of Active Tuberculosis after Failure of Previous Chemotherapy.** *Amer. Rev. Respiratory Dis.* 1959, July, v. 80, No. 1, Pt. 1, 89–94.

The emphasis which has been placed on cycloserine toxicity has prevented any large group of patients being treated as out-patients. A group of 56 middle-aged patients who were failures on standard treatment and who had refused further hospital treatment were selected. All were advanced cases. A daily dosage of 500 mgm. cycloserine and 300 mgm. of isoniazid was used. All had positive sputa; later the dose of cycloserine was increased to 750 mgm. daily. Unfortunately, sensitivity tests were not available.

41 patients completed 12 months, 4, 10 months, and 7, 6–9 months of treatment. It is reported that 1 patient had convulsive seizures and had ankle oedema and died before the cause could be investigated, 1 had

myoclonic involuntary movements, but these ceased when the dose of cycloserine was reduced; 1 schizophrenic patient had a mental relapse, and 1 had persistent hiccough. 26 patients had drowsiness and giddiness not sufficient to necessitate stoppage of the drug.

11 (21%) converted their sputum. 3, 4 and 5 patients showed marked, moderate or slight X-ray improvement respectively. About half the patients had clinical improvement, but the extent was different to assess.

The author concludes that the use of cycloserine for out-patients in the doses used was not very risky, and some benefit could be hoped for in a proportion of patients. *F. H. Young*

United States Pub. Health Service: **Tuberculosis Therapy Trial. Hepatic Toxicity of Pyrazinamide used with Isoniazid in Tuberculous Patients.** *Amer. Rev. Respiratory Dis.* 1959, Sept., v. 80, No. 3, 371–87. 3 figs. [15 refs.]

Pyrazinamide has been found to be very efficient in eradicating tubercle bacilli from the tissues of experimental animals when given in combination with isoniazid; favourable reports have been given by some authorities on human beings. Optimism over these results was clouded by persistent reports of the occurrence of hepatic damage in patients receiving pyrazinamide [this *Bulletin*, 1952, v. 27, 922; 1954, v. 29, 1040; 1955, v. 30, 24.]. Differences in the reported frequency and severity of hepatic disturbance emphasized the need for trial which will include a large number of cases, a concurrent control group, and frequent and systematic testing of hepatic function. From September, 1954, to March 1955, 780 patients had been selected for a trial undertaken by the United States Public Health Service Tuberculosis Committee. These patients were divided into 5 groups as far as possible matched; for special reasons the numbers in each group varied from 115 to 194. Between 13% and 20% of the patients had not previously received chemotherapy. Between 94 and 96% of the patients had evidence of cavitation. The 5 groups were subjected to 5 different regimes.

Regime

1. Isoniazid + PAS .. .. 24 weeks.
2. Isoniazid + pyrazinamide (25 mgm. per kgm.) 24 weeks.
3. Isoniazid + pyrazinamide (25 mgm. per kgm.) 12 weeks.
   Isoniazid + PAS .. .. 12 weeks.
4. Isoniazid + pyrazinamide (40 mgm. per kgm.) 24 weeks.
5. Isoniazid + pyrazinamide (40 mgm. per kgm.) 12 weeks.
   Isoniazid + PAS .. .. 12 weeks.

616 patients completed their assigned therapy, and 645 were available for evaluation of toxicity. Liver function tests were done on all patients whether they were receiving pyrazinamide or not. 1-minute and total serum bilirubin and cephalin cholesterol flocculation were measured at weekly intervals during treatment. Bromsulphthalein retention at 45 minutes with 5 mgm. of dye per kgm. of weight, was tested at the beginning and end of treatment and when other test values became abnormal. Hepatic toxicity developed in 21 patients, 5 of whom completed therapy; 16 were removed from their assigned regimes. Only 1 patient assigned to regime 1 (isoniazid-PAS) had any hepatic disturbance; he may have had pre-existing liver damage. On regimes 2 and 3 between 2 and 3% of the patients developed hepatic toxicity, 6% of liver toxicity appeared in patients on regime 4; only the difference between regimes 1 and 4 is statistically significant. Brief descriptions of the 21 hepatic toxic episodes are presented, grouped according to the severity of the toxic reaction. Hepatic toxicity was not shown to be associated with certain characteristics of the patients. Although no patient included in the trial had a history of chronic hepatic disease there were frequent abnormal pre-treatment values. Results in 28% of the patients were above the 0·2 mgm. per 100 cc. usually regarded as normal in the 1-minute serum bilirubin test. 6% of the patients had total serum bilirubin value above the recognized normal. 24% of patients had abnormally high pre-treatment bromsulphthalein tests and 29% of patients had abnormal pre-treatment cephalin flocculation tests. Serial examinations suggested that there is no general tendency for test values to increase as treatment continues. Opportunity was taken to examine the radiological changes observed after 24 weeks of treatment. Improvement was noted in 10 to 18% of patients in the various groups, but the difference is not statistically significant. The conclusion reached was that in the toxicity trial among treatment failures, a short course of pyrazinamide was relatively non-toxic, but the results in this period were not impressive.

This appears to be a very well conducted trial and the results are of considerable interest. *F. H. Young*

Romer, H. K. **Resection Treatment of Tuberculomas and Cancer-Suspect Tuberculous Lesions. Primary and Late Results.** *Acta. Tuberculosea Scandinavica.* 1959, v. 37, No. 4, 333–48. [49 refs.]

"Resection has proved to be a treatment giving good results in cases of tuberculomas. 26 patients have undergone resection; only one of them is still bacillary. No deaths.

"The time of observation was 5–9 years in the case of the 25 patients. The last patient, who could not be traced, was non-bacillary after observation for two years.

"22 of the 25 patients followed up have a full capacity for work and one has a partial capacity.

"10 of the above-mentioned patients have been operated upon as malignancy could not be excluded. Besides information is given of three patients who have undergone operations because of suspicion of cancer due to non-tuberculoma-like picture.

"All the 13 are non-bacillary after 5–9 years' observation, 12 have full capacity for work, and one is doing part-time work in another occupation."

KAPLAN, C. J. **Conservative Therapy in Skeletal Tuberculosis: an Appraisal based on Experience in South Africa.** *Tubercle.* 1959, Oct., v. 40, No. 5, 355–68, 8 figs. [15 refs.]

Mass radiography surveys have revealed that 1% of the African population of South Africa have active pulmonary tuberculosis [this *Bulletin*, 1956, v. 31, 502], and if 10% of these have skeletal tuberculosis as well there are about 10,000 persons who require orthopaedic treatment. The paucity of hospitals with operating facilities imposes the practice of conservative treatment.

Most of the patients are children under 10 years of age. The proportion of males to females is 3:2. In spite of the prevalence of cattle tuberculosis in South Africa bovine tubercle bacilli are rarely isolated from the patients [*ibid.*, 1954, v. 20, 523].

The spine is affected in about 70%, and the disease is far advanced in many cases when the patient first attends hospital. Advanced disease is also the rule in other forms of bone and joint tuberculosis. Nearly every patient shows evidence of active or, more rarely, healed pulmonary tuberculosis. Many of the children have miliary disease. Malnutrition is common.

Rest, adequate nutrition, hygienic surroundings and chemotherapy are the basis of treatment. Streptomycin and isoniazid are given but PAS is not used routinely. The need for giving vitamin B complex and nicotinic acid is mentioned. Rigidly enforced rest is not the rule. The aim is to avoid weight-bearing during the active phase of the disease. Children are nursed prone and held by a canvas corset; leg traction is used. Adults are merely kept in bed. In disease of the cervical spine the head is immobilized by a collar.

Most patients improve rapidly after admission to hospital; the destructive process is arrested within 6–9 months in most cases, and healing by fusion of the affected vertebrae takes place in about half. Operative spinal fusion is done where indicated and where possible.

Abscesses are left alone unless presenting or causing pressure symptoms. If necessary they are incised, evacuated and irrigated with a mixture of streptomycin and isoniazid. Healing without sinus formation is usual.

When recumbency is no longer required the patient is sent home wearing a spinal support. He continues to take isoniazid for 1 year. Only a few patients require spinal fusion at the end of that period.

The results obtained in patients treated before and after 1950 were analysed. It was seen that the use of chemotherapy shortened the duration of hospital treatment and reduced both the death and relapse rates. The extent of spinal involvement was also reduced and bony fusion occurred in a higher percentage of the patients. The incidence of paraplegia before and after 1950 was much the same but the results in such patients were greatly improved. Intra-articular injection of a mixture of streptomycin and isoniazid shortened the course of the illness in joint disease.

The author's conclusion is that in skeletal tuberculosis conservative treatment is to be recommended in the first instance especially in such conditions as those obtaining in South Africa. *H. G. Calwell*

FYFE, W. M. **Treatment of Tuberculous Meningitis.** *Arch. Dis. in Childhood.* 1959, Aug., v. 34, No. 176, 334–7.

When isoniazid is used for chemotherapy in tuberculous meningitis the need for intrathecal therapy is controversial. An excellent opportunity of deciding whether intrathecal therapy is essential arose when in February 1954 routine intrathecal therapy was abandoned by one medical unit in the Royal Hospital for Sick Children, Glasgow, but retained by the other. The author discusses 40 cases of tuberculous meningitis admitted to the Royal Hospital for Sick Children, Glasgow between 1 February 1954, and 31 December 1957. 20 patients were treated with intrathecal therapy, and 20 without. The table shows that the age distribution and stage of the disease of the patients treated by the 2 methods were roughly comparable.

Group I (with intrathecal therapy) were treated with doses of intrathecal streptomycin ranging from 25 to 50 mgm. per injection according to age. Injections were given daily for a week, and after this on alternate days twice weekly or weekly according to the physician's appraisal of the patient's progress. Intramuscular streptomycin was given by a daily dose of 20 mgm. per lb. (40 mgm. per kgm.) body weight for 3 to 7 months. Oral isoniazid in a dose of 10–20 mgm. per kgm. body weight was given for 5 to 10 months in 15 cases and for 3 to 4 months in 4. The remaining patient died in the 5th week of treatment.

In Group II (without intrathecal therapy) the same regime as regards intramuscular streptomycin and oral isoniazid was given. Additional drug therapy in the form of PAS or steroids was given to a few patients in each group. 17 patients in Group I, and 15 in Group II survived; 14 of the survivors in each group are well and have no sequelae. 5 patients in Group I had a relapse, in 3 of these antituberculous drugs were given initially for less than 6 months. No patient in Group II has relapsed. Of the 5 patients in Group II who died, 2 would almost certainly have done so under any treatment.

It is generally accepted that, apart from the nature of the therapy used, the most important factor governing the prognosis in tuberculous meningitis is the stage of the disease at the start of treatment. All patients in both groups with advanced disease either died or had residual disabilities. 74% in Group I, and 82% in Group II, of patients in the early and medium stages made a complete recovery. In neither group did an associated miliary tuberculosis of the lungs adversely affect the prognosis. The authors conclude that daily intramuscular injections of streptomycin for 6 months, and daily oral isoniazid for a similar or even longer period is the treatment of choice. *F. H. Young*

DORMER, B. A., HARRISON, I., SWART, J. A. & VIDOR, S. R. **Prophylactic Isoniazid. Protection of Infants in a Tuberculosis Hospital.** *Lancet.* 1959, Nov. 21, 902–3, 1 fig.

At King George Vth Hospital, Durban, South Africa, it was the custom, until 1955, to separate newborn

babies immediately from mothers who had active tuberculosis, and send them to other institutions, foster-parents, or relatives. The mortality rate was dreadful. Since that time, no effort has been made to separate the children from their mothers or take any precautions against infection, and breast feeding has been the rule. From birth to 6 months, the children were given 25 mgm. of isoniazid in a syrup, twice daily, and after 6 months the dose was increased to 50 mgm. twice daily. No adverse effects have been noted.

Between October 1955 and January 1959 there have been 101 confinements and 103 babies born. 5 were stillborn, or died within the first 3 weeks of life. The remaining 98 were kept in hospital for 1 to 22 months, the average stay being 6 months. 4 of these children became Mantoux positive, but it was discovered that the mothers were not giving the isoniazid to the children regularly. It was therefore decided that nurses should give the children isoniazid, and since then no other child has become Mantoux positive while in hospital.

Regular follow-up has been impossible, but 22 of the children have been seen as out-patients, of whom 3 have become Mantoux positive since discharge after their mothers had stopped giving them isoniazid.

Of the mothers, 2 have died, 1 from haemoptysis, and 1 from cor pulmonale. The disease appeared to behave as would have been expected in women who were not pregnant. BCG was not given, as the conversion of the Mantoux test was the most important method of detecting infection. In future, however, it is intended to give isoniazid resistant BCG to all children before they leave hospital. Where isoniazid has been given regularly it has completely prevented tuberculous infection in the children, but no immunity has, of course, developed.

[This is a very valuable paper, and confirms work by other authors on this subject. It suggests that the practice of separating children from their tuberculous mothers at birth will have to be revised in almost all countries.] *F. H. Young*

LAMBERT, H. P. **The Chemoprophylaxis of Tuberculosis. A Review.** *Amer. Rev. Respiratory Dis.* 1959, Nov., v. 80, No. 5, 648–58. [37 refs.]

MEDICAL RESEARCH COUNCIL. **B.C.G. and Vole Bacillus Vaccines in the Prevention of Tuberculosis in Adolescents. Second Report to the Medical Research Council by their Tuberculosis Vaccines Clinical Trials Committee** [HART, P. D'A., Chairman]. *Brit. Med. J.* 1959, Sept. 12, 379–96, 2 figs. [29 refs.]

A clinical trial of BCG and vole bacillus vaccines directed by the Tuberculosis Vaccines Trials Committee of the Medical Research Council of England and Wales was begun in England in 1950. The participants were schoolchildren aged 14½–15 years at the time of entry to the trial.

The first report was published in 1956 when each participant had been in the trial for 2½ years. It was the subject of a long notice in this *Bulletin* [1956, v. 31, 621] which included details of the plan and conduct of the trial and the safeguards that were adopted against bias.

The present report continues the story and presents results for all who have been in the trial for 5 years with incomplete information up to 7½ years.

Follow-up continued to be highly successful, sample analysis showing that some 90% of the participants were reached during the years 1957 and 1958. Only 1 died of tuberculosis within 5 years of entry. He was an unvaccinated non-reactor and the cause of death was tuberculous meningitis.

Cases of tuberculosis starting within 5 years of entry to the trial were:

Section*		Trial Group	Estimated No. of Participants	Definite Cases of Tuberculosis		Possible Cases of Tuberculosis Starting Within 5 Years
				No. Starting Within 5 Years	Annual Incidence per 1,000 Participants	
A	Children admitted concurrently with those given BCG vaccine	Negative unvaccinated	13,200	151	2·29	9
		Negative, BCG vaccinated	14,100	27	0·38	3
		Positive to 3 TU	15,800	127	1·61	6
		Positive only to 100 TU	6,500	30	0·92	3
B	Children admitted concurrently with those given vole bacillus vaccine	Negative unvaccinated	6,500	85	2·62	7
		Negative, vole bacillus vaccinated	6,700	11	0·33	2
		Positive to 3 TU	8,800	76	1·73	3
		Positive only to 100 TU	3,600	17	0·94	2
C	Children admitted concurrently with those given BCG and those given vole bacillus vaccine	Negative, BCG vaccinated	6,400	10	0·31	2
		Negative, vole bacillus vaccinated	6,400	8	0·25	2

*Many participants and cases of tuberculosis appear in more than one of the three separate sections of this table and the figures from different sections can therefore not be totalled.

349 cases of definite tuberculosis were found, and 23 of possible tuberculosis. Of the definite cases, 153 occurred in the negative unvaccinated group, 27 in the BCG group, 11 in the vole bacillus group, 128 among original reactors to 3 TU and 30 in original reactors only to 100 TU.

As the value of vaccination can only be assessed validly by comparing results among children admitted concurrently to the trial, the data are presented as set out in the above table.

The incidence of tuberculosis in each trial group was closely similar in the different sections of the table, and because of the homogeneity of the findings it was considered unnecessary to set out all the results on a strictly concurrent basis and so findings are later presented for the whole of each trial group.

Counts of viable bacilli were made as a routine on each weekly batch of vaccine in Copenhagen before dispatch to England. It was thus possible to investigate whether the post-vaccinal tuberculin conversion rate or the incidence of tuberculosis was associated with fluctuation in the strength of the vaccine.

The total conversion rate with BCG vaccine was practically 100% but the rate of reaction to 3 TU increased with an increasing bacillary count from 76%, when the count was under 20 million viable units per mgm., to 97% when it was 40 million or more. There was no observed trend in the degree of protection afforded by vaccine of different strengths. The weakest batches conferred substantial protection.

Similar observations were made with vole bacillus vaccine. Early in 1951 the vaccine in use was weaker than intended and the post-vaccinal conversion rate (3 TU) was only 31% with a total conversion rate of 87%. Later vaccine was of standard strength and gave corresponding conversion rates of 88% and 100%.

Despite this marked difference in conversion rates with the weaker and the stronger vaccine there was no substantial difference in the degree of protection afforded by any of the batches of vole bacillus vaccine.

Evidence of technically satisfactory vaccination was sought in the cases of the 38 vaccinated children who developed definite tuberculosis. 21 were found to be reactors within 6 months of vaccination and 5 more than 6 months after vaccination. Of these 26, 20 were found to have in addition a healed vaccination lesion, in 2 the vaccination site was not examined, and in 4 (all vaccinated with vole bacillus vaccine) no vaccination lesion was found on examination at 4, 6, 6 and 12 months after vaccination.

In 12 cases there were no tuberculin tests after entry and before the disease developed but 10 had a healed vaccination lesion. It was concluded that 2 of the children may not have been vaccinated satisfactorily by the usual criteria.

The forms of tuberculosis starting within 5 years of entry were:

Trial Group	Total Cases	Pulmonary Tuberculosis		Tuberculous Pleural Effusion*	Hilar Gland Enlargement†	Tuberculous Meningitis	Bone or Joint Tuberculosis	Tuberculous Cervical Adenitis	Tuberculous Peritonitis	Erythema Nodosum	Other Forms
		No.	%								
Negative unvaccinated ...	153	100	*65*	36§	2	4	2‡	3	2‖	3	1¶
Negative, BCG vaccinated	27	20	*74*	5	0	0	0	0	1	1	0
Negative, vole bacillus vaccinated	11	7	*64*	3	0	0	1	0	0	0	0
Positive to 3 TU	128	97	*76*	9	0	1	3	11	1	0	6**
Positive only to 100 TU	30	21	*70*	6	0	0	2	0	0	0	1††
All groups ...	349	245	*70*	59	2	5	8	14	4	4	8

*Without evidence of pulmonary tuberculosis. †Without other evidence of tuberculosis. ‡One with erythema nodosum. §Three with erythema nodosum. ‖One with small associated pulmonary lesion. ¶Tuberculous bronchiectasis. **One tuberculous endobronchitis; one tuberculous tonsils and cervical glands; one lupus vulgaris; three genito-urinary tuberculosis. ††Tuberculous axillary adenitis.

70% of the cases (245 of 349) were of pulmonary tuberculosis. The nature and extent of the lesions seen in the first abnormal chest skiagram are tabulated:

Trial Group	Total Pulmonary Cases	Miliary Type of Lesions	Lesions with Cavitation			Lesions without Cavitation		
			Lesions Involving More than 2 Rib Interspaces	Lesions More than 6 sq. cm. in Extent Involving Up to 2 Rib Interspaces	Lesions Up to 6 sq. cm. in Extent	Involving More than 2 Rib Interspaces	More than 6 sq. cm. in Extent Involving Up to 2 Rib Interspaces	Up to 6 sq. cm. in Extent
Negative unvaccinated	100	4	18	6	1	4	36	31
Negative, BCG vaccinated	20	0	2	2	1	0	6	9
Negative, vole bacillus vaccinated	7	0	1	0	0	0	3	3
Positive to 3 TU	96*	1	8	7	1	8	37	34
Positive only to 100 TU	21	0	1	3	0	1	11	5
All groups	244*	5	30	18	3	13	93	82

*Excluding one patient for whom the extent of the lesions was not classified because the first abnormal radiograph, taken before a thoracoplasty was performed, could not be found; the diagnosis was confirmed bacteriologically.

The figures suggest (without being statistically significant) that the disease was on the average rather less extensive in the vaccinated than the unvaccinated children.

The serious nature of the illness is shown by the finding that the physicians in charge of the patients [who had nothing to do with the trial] took 243 of them (70%) away from work and 222 of these received chemotherapy or were treated by collapse or surgically.

During the first 2½ years the annual incidence of tuberculosis in the negative unvaccinated group was 1·98 per 1,000, in the BCG group 0·43 and in the vole bacillus group 0·42. The incidence in the combined vaccinated groups was only 21% of that in the unvaccinated non-reactor group.

During the second 2½ years the corresponding rates were 2·62, 0·34 and 0·24 per 1,000 respectively. The total incidence in the combined vaccination groups was only 12% of that in the negative unvaccinated group. The incomplete figures for the third period to 7½ years show that the incidence in the vaccinated group was only 24% of that in the negative unvaccinated group.

"Among those with strong positive reactions to 3 T.U. on entry (15 mm. induration or more) the annual incidence of tuberculosis was 3.50 per 1,000 in the first two and a half years, 1.67 in the second two and a half years, and 0.88 in the (incomplete) five to seven-and-a-half year period. In contrast, the annual incidences among those with weaker positive reactions to 3 T.U., and among those positive only to 100 T.U., were respectively 0.77 and 0.73 per 1,000 in the first two and a half years, and remained at much the same level thereafter. Thus, in this age group, those highly sensitive to tuberculin had a special risk of developing tuberculosis during the following few years. Those with lesser sensitivity to tuberculin on entry had consistently lower rates than those in the negative unvaccinated group, suggesting that they had some degree of protection against fresh infection, though not as great as that in the vaccinated groups.

"In assessing the benefit that would have accrued to the entire trial population from the use of vaccine for *all* those tuberculin negative on entry, the contribution to the total tuberculosis morbidity made by those initially tuberculin positive (and therefore ineligible for vaccination) had to be included. This benefit—that is, the percentage reduction in incidence of tuberculosis during the five-year period—was 59% for B.C.G. vaccine and 61% for vole bacillus vaccine.

"In all, 125 participants died from causes other than tuberculosis within five years of entry to the trial. In addition 151 cases, when submitted to the assessor, were classified by him as not due to tuberculosis, these consisting mainly of other chest diseases submitted because the radiographic abnormality persisted for more than 14 days. The incidence of these non-tuberculous deaths and diseases in the five trial groups reveals no significant evidence of protection by B.C.G. or vole bacillus vaccination against any specific cause of death, nor against any chest disease other than tuberculosis.

"The trial is still in progress, and later reports will contain more detailed analyses over longer periods of time." *H. G. Calwell*

WELLS, A. Q. & WYLIE, J. A. H. **Intradermal Vaccination with the Vole Bacillus.** *Tubercle.* 1959, June, v. 40, No. 3, 181–3. [25 refs.]

The murine type of *Mycobacterium tuberculosis* (vole bacillus) has been used as an immunizing agent against tuberculosis in man by a number of workers. The vaccine has been shown to be as efficient as any other so far used.

Most of the vaccinations done by the authors and all those carried out in the course of the Medical Research Council's trials [this *Bulletin*, 1956, v. 31, 621] were by multiple puncture. Lupus developed occasionally at the site of vaccination, and it seemed possible to the authors that the higher incidence of lupus following vaccination with the vole bacillus than with BCG might be due to the larger area of skin involved in multiple puncture than in intradermal vaccination. Also the higher incidence of lupus might be due to the greater virulence of the strain of vole bacillus used compared with BCG.

Accordingly a trial was carried out in which 172 persons were given a dilution of vaccine prepared for multiple puncture vaccination. Administration was by intradermal injection. Each person received 0·00001 mgm. or 0·000025 mgm. moist weight of bacilli in 0·1 ml. The vaccine was not unicellular, some of the clumps of bacilli containing as many as 50. The local reactions were small, most being less marked than after vaccination with BCG. 2 axillary gland abscesses occurred and one slight lupus reaction which was not treated. Tuberculin conversion was satisfactory, being 100% at 7 months, and 79% of those vaccinated were still reactors at 41 months (Mantoux 100 TU).

A method of vaccine production was finally adopted which produced a suspension containing at least 70% single bacilli and no clumps of more than 10 cells. This vaccine was given by intradermal injection to 385 persons. The local reaction was negligible, there were no gland abscesses and lupus did not occur. Bacillary count showed that the dose given to each person was 25,000 to 50,000 bacillary units. Tuberculin conversion occurred in 100% in 4–8 weeks. The reactor rate was 95% at 10–13 months and 84% at 22 months (Mantoux 100 TU).

The conversion rates and the persistence of tuberculin sensitivity compared favourably with those following BCG vaccination. [A footnote records that Wells died in October 1956.] *H. G. Calwell*

DAHLSTRÖM, G. **BCG Vaccination in Sweden.** *Tubercle.* 1959, June, v. 40, No. 3, 196–200. [20 refs.].

BCG vaccination has long been practised in the Scandinavian countries as a valuable part of tuberculosis prevention.

In recent years more than 90% of the children in Stockholm (Sweden) have been vaccinated soon after birth or before entering school so that in 1956 92% of school entrants (at 7 years) had already been vaccinated and were tuberculin reactors, while 2% had been vaccinated but were found to be non-reactors. Slightly

more than 1% were reactors but were not known to have been vaccinated, and less than 5% were non-reactors and unvaccinated. Of the few non-reactors the great majority were vaccinated during their first year in school. A similarly high rate of vaccination was reported from another region of Sweden.

The Swedish programme also comprises vaccination or re-vaccination of non-reactors at about 14 years of age. The members of the armed forces, medical personnel and, most important, contacts of tuberculous persons are all offered vaccination.

The need for the continuation of mass vaccination in Sweden has been investigated by the Swedish Association of Chest Physicians and a report was issued in 1958. The recommendations made are as follows:

Vaccination of healthy full-term infants should be continued *for the present*. Post-vaccinal tuberculin tests need only be done where there is contact with a tuberculous person. The vaccination of non-reactor school entrants may be restricted without risk of serious consequences.

All schoolchildren should be offered vaccination at 10–11 years rather than later, as vaccination about the time of puberty confers reliable protection against tuberculous disease in adolescence or early adult life. Propaganda to this effect is desirable.

Vaccination of conscripts, university students, seamen, medical personnel and contacts of all ages should be continued.

Dahlstrom adds his personal reflections on the foregoing. If BCG vaccination is effectively carried out in schools young persons entering industry will be well protected against infection.

The loss of the tuberculin test as an epidemiological tool is not serious as this test is not in any case used on a sufficiently wide scale in Sweden for it to be effective; moreover it is not infallible.

The presence of BCG-induced tuberculin sensitivity may occasionally impede diagnosis but Swedish chest physicians have not objected to BCG on this score.

The preponderance of post-primary pulmonary tuberculosis, which occurs in persons who are reactors following infection many years earlier does not argue against BCG vaccination but in favour of vaccinating at an age when only a few persons have become naturally infected. The frequency of serious complications, including fatalities is much too small to be a decisive argument against vaccination.

H. G. Calwell

LAHESMAAA, R. [Edited by.] **Proceedings of the Nineteenth Scandinavian Congress for Pulmonary Diseases in Turku (Abo), August 7–9, 1958.** *Acta Tuberculosea Scandinavica.* 1959, Suppl. 45, 134 pp., illustrated. [Numerous refs.]

The 19th Scandinavian Congress for pulmonary diseases held on August 7–9, 1958, devoted its first 2 sessions to hearing correlated collections of the papers on sarcoidosis, and rehabilitation of patients suffering from pulmonary tuberculosis or its effect. The remaining 2 sessions were devoted to a series of papers mainly on unrelated subjects dealing with various aspects of lung disease.

The first morning session was devoted to the rehabilitation of tuberculous patients in Scandinavia. L. ERKKI, pp. 45–48, pointed out that in Finland, where treatment is successful in a high percentage of cases, it is the public fear of infection that is the main reason for any differentiation between rehabilitation of the tuberculous and other long term convalescents; he considered that 10% of patients need special institutional rehabilitation. J. PATIALA, pp. 49–55, said that 2 main groups required attention: (1) the lung invalids no longer infectious, and (2) the chronic or unhealed patients. In Finland the first group are dealt with in special vocational schools of which there are 3 under the Ministry for Social Affairs. In Finland so far little provision has been made for the second group.

The work of the rehabilitation sections of the Satakunta sanatorium is described by R. ELO, pp. 66–74. From 1933 to 1957 1,451 patients have been discharged from there, of whom 1,052 were labourers.

T. HELMS, pp. 56–61, and K. ISAGER, pp. 62–65, described the methods in vogue in Denmark, the former at Nakkebølle Sanatorium, and the latter in the rehabilitation in the city and county of Aalborg, Denmark. These papers are interesting from the point of view of the work in different countries, but contain little that is very original.

Differential ventilatory function of the two lungs is usually estimated with the patient at rest; N. RISKA and T. L. SELLERGREN, pp. 77–82, point out that it is more rational to do the estimation during exercise, and used bronchospirometry in conjunction with ergometry in the recumbent position on 17 patients. For the less ventilating lung, spirometry recorded values of 0–35% of the total oxygen uptake, and 0–45% of the total ventilation. There was considerable augmentation of the ventilation and O_2 uptake in the less efficient lung on mild exercise both relatively and absolutely, but as exercise rose although the O_2 uptake rose still further the percentage fell.

N. SVANBORG, p. 76, in an abstract maintains that the results of pleurectomy are better than one usually realized. An interesting paper by A. PEKKARINEN, K. LYYTIKÄINEN & M. TURUNEN, pp. 83–96, deals with the question whether in advanced pulmonary tuberculosis there is a decrease in the adrenocortical reserve. This might be expected, and during the first 3 days following big lung operations there is an increased output of 17-hydroxycorticosteroids (17-OHCS) which is of some importance. Several papers published recently have reported the value of hydrocortisone for tuberculous patients who have collapsed after lung operations. In 104 patients the authors found that there was no uniform diminution of the 17-OHCS reserve, nor was their reaction to the use of 80 units Corthrophin Z [corticotrophin], depot intramuscularly significantly altered. Their figures suggest a drop in the reserve in some patients, but no common factor explaining this is obvious.

Among papers dealing with surgical subjects, one by S. J. VIIKARI, V. AUTIO and P. ANTILA, pp. 124–130, on 137 patients treated by extrapleural pneumolysis and followed up for 3 years, is interesting as this method of treatment was largely abandoned in Great Britain because of complications. The introduction of

antimicrobials has completely changed the situation, but the fear of complications persists. 90% of patients are well and working; 4 patients convalescent, 2 died, and 7 still have a positive sputum although the reason is not stated.

The final session of the meeting was largely concerned with antimicrobial drugs, especially PAS, but several appear only as summaries. A. HANNGREN, p. 115, using tritium as a tracer, found that intracellular PAS was present in cartilage and connective tissue. It accumulated in tissue changed by tuberculosis, of a proliferative nature. In resected specimens poor concentration was found in cavity contents, but a high concentration in cavity walls.

H. O. BANG and E. STRANDGAARD, p. 116, found that aminosalyl calcium in doses of 12 gm. daily gave maintained serum concentration of 3–4 mgm. to 12–14 mgm. per 100 ml., while the figures for a similar dose of aminosalyl sodium were 2–4 mgm. up to 8–10 mgm. per 100 ml. Phenyl PAS gave capricious results. J. LEHMANN, p. 121, commenting on these and other papers, said that if high doses of PAS were given, the acetyl conjugating enzyme is inactivated with the result that a double effect is produced—more PAS in the serum and less PAS inactivated. He condemned the use of slowly absorbed compounds.

A. WESTERGREN, p. 122, [only summarized] emphasized that the symptoms of intolerance could not be equated with an allergic reaction.

[This report is a little disappointing, apart from the discussion on sarcoidosis in which the Scandinavian countries are very interested. A factor in this criticism is that several of the papers delivered which gave promise of being interesting are summarized very shortly as their publication is promised elsewhere.]

F. H. Young

VENEREAL DISEASES

BROWN, W. J. SELLERS, T. F. & THOMAS, E. W. **Challenge to the Private Physician in the Epidemiology of Syphilis.** *J. Amer. Med. Ass.* 1959, Sept. 26, v. 171, No. 4, 389–93, 5 figs.

It is an accepted fact that general practitioners treat a certain proportion of patients with syphilis but do not notify them to the authorities. Syphilis rates fell steadily in the United States between 1947 and 1954 but since then have remained steady. Failure of the downward trend can hardly be due to decreased public health activities but is probably, partly at least, due to more patients going to private practitioners; it is estimated that only about 25% of these are reported to the health authorities.

It is calculated that if a careful follow-up of the 12,028 cases of infectious syphilis which *were notified* by private physicians had been carried out, 2,868 cases of primary and secondary, 1,606 of early latent and 594 of "other" syphilis would have been discovered.

The suggestion is that, if private physicians would notify the health authorities, trained personnel would interview patients, trace contacts and bring them to examination and, if necessary, treatment without further trouble to the doctor. The difficulty is to induce the doctor, who is usually a very busy person, to notify cases but the authors of this paper believe it can be done.

T. E. Osmond

DENCKER, F. & NIELSEN, H. A. **The Incidence of Syphilitic Infection in Individuals aged 65 and 70 Years in Bornholm demonstrated by History and by Clinical and Serological Examination.** *Danish Med. Bull.* 1959, Oct., v. 6, No. 7, 230–39. [18 refs.]

These authors investigated 882 persons aged 70 and 65 years in Bornholm for the presence of syphilis. Serum reactions consisted of the Wassermann (C-WR-M [Cardiolipin-Wassermann Reaction-Mørch modification]), Kahn and Meinicke tests and the TPI test; histories and clinical examinations were also included; advantage was taken of the syphilis register maintained at the Serum Institute.

Results are set out in detail in the text and in 6 tables. The article is long and somewhat complicated.

There is evidence that 17 of the 882 persons had been infected with syphilis at some time; of these 16 gave positive TPI reactions and 14 positive classical serum (STS) reactions; in only 5 were both types of reaction positive; in only 1 case was a positive TPI reaction suspected of being false whereas several of the STS were undoubtedly so. The results recorded in the syphilis register must be considered unreliable since they were entirely dependent on the STS. Taking TPI results as true there was no difference in the incidence of syphilis between males and females and between the 2 age-groups; clinical investigation revealed 1 case of syphilitic aortic disease and 1 with Argyll-Robertson pupils; 5 patients had cardiovascular symptoms probably due to arteriosclerosis.

This investigation suggests that syphilis is common in ports, tends to affect a low socio-economic group and that in Denmark many cases are imported.

T. E. Osmond

RAGAB, A. F. **A Comparative Study of the Incidence of Venereal Diseases in Egypt and their Treatment.** *J. Egyptian Med. Ass.* 1959, v. 42, No. 1, 1–8.

After the introduction of penicillin there was a steady fall in the incidence of early syphilis and gonorrhoea. Recently this fall has stopped and non-gonococcal urethritis has even tended to increase.

To combat syphilis this author recommends (1) mass

improved serum testing, (2) treatment of all cases found, (3) follow-up for a year after treatment, (4) treatment of contacts [? whether infected or not], (5) allocation of funds for health education, (6) encouragement for doctors to specialize in VD and (7) continued research in VD.

The treatment of syphilis should be with penicillin—PAM 600,000 units every 2–3 days for 10 injections or benzathine penicillin given weekly, every 2 weeks or every 3 weeks; arsenic and bismuth may be given as well [but few syphilologists employ these nowadays]. Gonorrhoea is becoming more and more resistant to penicillin and the broad-spectrum antibiotics may be necessary. Non-gonococcal urethritis may be due to any of a number of organisms or to a virus; treatment with antibiotics is only partially successful. Trichomoniasis appears to be increasing and trichomycin is employed in treatment.

Lymphogranuloma venereum is often overlooked; for treatment chloramphenicol, erythromycin or tetracycline are used. Granuloma inguinale is rare; wide-spectrum antibiotics give the best treatment results. Chancroid also is rare and reacts well to similar treatment.

In a table is set out the percentage of sero-positive cases in various parts of Egypt over the 5 years 1952–1956; Suez, Port Said and Aswan shows the highest figures in 1956. *T. E. Osmond*

ENG, J. Forekomsten av seropositiv syfilis blant svangre Norske kvinner. 1953–1958. [**The Occurrence of Sero-Positive Syphilis among Pregnant Norwegian Women**] *Tidsskr. f. d. Norkse Laegeforening.* 1959, Dec. 1, v. 79, No. 23, 1361–4, 2 figs. [16 refs.]

There has been a considerable fall in the number of notifications of new cases of syphilis in Norway since the war, and they fell from 1,784 in 1946 to 130 in 1957. There is also a considerable fall in the number of positive serological reactions in the blood of pregnant women sent to the Sero-Diagnostic Department of the State Institute of Public Health in Oslo to which some 38,000 samples of blood from pregnant women are sent yearly in search for syphilis and for rhesus-typing. Among the 179,440 women thus examined during the 5-year period 1953–58 were 848 who gave positive syphilis reactions. This occurred at the rate of 0·47% for the whole period. It fell from 0·63% in the first year to 0·34% in the last year. For women aged over 30 the syphilis rate was 0·62%, whereas for women under this age this rate was down to 0·18%—a finding which Eng interprets as reflecting on the spread of syphilis in Norway during the war. At his laboratory the blood of pregnant women undergoes 3 serological tests—Meinicke II (MKR), Bordet-Wassermann (WaR), and the Wadsworth and Brown flocculation reaction (W-Br.). This last reaction has been replaced since the beginning of 1959 by the VDRL flocculation reaction. To distinguish between syphilitic and non-syphilitic positive reactions, Eng has learnt to depend much on the TPI test after 3 years of the routine employment of it. Thanks to it about one-fifth of the positive reactions have been found to be false. Should there be a further fall in the frequency of positive reactions, a relative rise in the frequency of such false positive reactions may be expected.

Claude Lillingston

DIETZ, O. & SCHNEEWEISS, U. Serologische Untersuchungen zur Frage der prognostischen Bedeutung des Nelson-Mayer-Testes für die Heilung der Syphilis. [**The Value of the Nelson-Mayer-Test in Assessing the Efficacy of Treatment of Syphilis**] *Ztschr. f.d.g. Hyg. u. Ihre Grenzgebiete.* Berlin. 1959, Sept.–Oct., v. 5, No. 5, 295–303. [19 refs.]

In this paper the authors refer to the difficulties which still exist in deciding whether or not a patient has been cured of a syphilitic infection. The introduction of the TPI test has given results which suggest that the criteria accepted hitherto of cure may be imperfect. Some description of and abstracts from the literature on these tests are given.

The authors' experience of the TPI test is fully described and discussed with some tables which set out the negative, doubtful and positive results obtained in patients with secondary syphilis who had been cured according to the findings of the classical serological tests. About 20% of these treated patients yield a positive TPI test and negative Wassermann tests but the significance of this requires much further study. It may be that the persistent positive TPI test is of no importance, or is an indication that further active treatment is required. In the meantime, however, these patients should remain under observation pending the results of further experience.

[The authors differentiate the "Nelson test (NT)" from the TPI test in the text, though without explanation, and then appear to use the two designations synonymously.] *M. E. Delafield*

KOSTANT, G. H., LANDY, S. E., MILLER, J. N. & KELCEC, Louise C. **Treponemal Tests on Cerebrospinal Fluid. Experiences with the *Treponema pallidum* Complement-Fixation (TPCF) and Reiter Protein Complement-Fixation (RPCF) Tests.** *Arch. Dermat.* 1959, Oct., v. 80, No. 4, 439–41.

A brief review of the literature indicates that the TPI test on the cerebrospinal fluid (CSF) is usually positive in central nervous system (CNS) syphilis and in about 25% of cases of latent syphilis.

These authors carried out the *Treponema pallidum* complement-fixation test (TPCF), the Reiter protein complement-fixation test (RPCF), the cardiolipin complement-fixation test (cardio CF) and in a few cases the TPI test on 187 cerebrospinal fluids.

In 96 fluids from presumably normal persons all tests were negative; of 42 from persons with latent syphilis 13% were reactive with TPCF, 4% with RPCF and none with cardio CF; of 49 from persons with CNS syphilis 91% were reactive with TPCF, 85% with RPCF and 73% with cardio CF; in 12 the TPI test was uniformly negative.

It is of interest to note that storage in deep-freeze tends to result in a positive CSF becoming negative.

These two treponemal CF tests appear to be highly specific and reasonably sensitive; their reactivity in latent syphilis is not surprising since it has long been known that many latent syphilitic persons suffer from asymptomatic neuro-syphilis.

What is surprising is that the TPI was negative in all 12 cases; presumably there was a technical error somewhere. *T. E. Osmond*

STEINMETZ, P. R. & GREEN, J. P. **Reiter's Syndrome. Report of 16 Cases, 8 of which followed Treatment for Gonorrhea.** *U.S. Armed Forces Med. J.* 1959, Oct., v. 10, No. 10, 1185–93. [15 refs.]

An account is given of 16 cases of this disease with full details of 2. 14 out of the 16 admitted sexual exposure and only 1 complained of diarrhoea; most started with urethritis followed by conjunctivitis and then arthritis; this last generally affected more than 1 joint, knees and ankles being the commonest; two-thirds of the patients showed muco-cutaneous lesions including balanitis circinata and keratodermia blenorrhagica.

Treatment consisted of physiotherapy, salicylates and a generous diet; cortisone, prednisolone or corticotrophin appeared rather more effective than salicylates; penicillin and streptomycin appeared ineffective. Recovery usually occurred in 2–5 months.

The cause is still unknown; these authors throw doubts on its venereal origin. The role of pleuropneumonia-like organisms, allergy, dysentery and L forms of bacteria is discussed; there is some evidence against an infective origin. *T. E. Osmond*

BURCH, T. A., REES, C. W. & REARDON, Lucy V. **Epidemiological Studies on Human Trichomoniasis.** *Amer. J. Trop. Med. & Hyg.* 1959, May, v. 8, No. 3, 312–18. [15 refs.]

An investigation was carried out on 469 women attending a gynaecological clinic in Washington, D.C., and on 1,848 female employees of the National Institutes of Health Bethesda, Maryland; 97 women found to be infected were studied in detail. Statistical results are set out in 4 tables.

Cultures from the vagina were made on trypticase-liver-serum; mouth cultures were placed in Locke-egg medium.

In the employee group Negresses were infected 8 times as often as White women but in the clinic group numbers were about equal; the age-group with the highest infection rate was 30–49 years.

Married and unmarried Negro women were equally affected but married White women in the employee group were more often infected than single ones; White Protestant women were more often infected than non-Protestant (*e.g.* Jewish and [Roman] Catholic) women.

Trichomonas vaginalis was associated with *T. tenax* very frequently which suggests they may be the same species.

Vaginal fungi were less common in Negresses than *T. vaginalis* but more common in White women.

Nearly 50% of husbands of infected women were also found to be infected and wives living with their husbands were much more difficult to cure than others; infected fomites yielded positive cultures of *T. vaginalis* in numerous experiments. *T. E. Osmond*

GENERAL INFECTIVE DISEASES

The order in this section is:—**General; Hospital and other Cross Infections; Coccal Infections; Bacterial Infections; Virus Infections.**

GENERAL

U. S. DEPT. OF HEALTH, EDUCATION, & WELFARE. **Immunization Information for International Travel. Prepared by the Epidemiology and Immunization Branch, Division of Foreign Quarantine of the Bureau of Medical Services.** *Pub. Health Service Publication No. 384.* Revised June 1959. 77 pp., 3 maps. Wash. 25: U.S. Govt. Printing Office. [$0.30.]

This is a revised edition of the publication reviewed earlier [this *Bulletin*, 1959, v. 34, 105].

The fifth and sixth sections have been brought up to date to June 1959.

TAYLOR, I. **Immunization Schedules.** [Correspondence.] *Brit. Med. J.* 1959, Oct. 31, 884.

If a child is not brought for immunization at the most suitable age for beginning the course, some modification of the programme becomes necessary. The author gives the following table which has been proved helpful.

Changes in the later columns are governed by the fact that as the child becomes older the urgency of whooping cough protection becomes less and that of poliomyelitis protection greater.

Age in Months	Course for a Child Presented for the First Time at the Age in Left-hand Column								
	a	b	c	d	e	f	g		h
0									
1									
2	Triple								
3	,,	Triple							
4	,,	,,	Triple						
5	Smallpox	Smallpox	,,	Triple	Smallpox				
6			Polio	,,	Polio	Polio	Polio		
7	Polio	Polio	,,	,,	,,	,,	,,		Polio
8	,,	,,	Smallpox	Polio	Triple	Smallpox	Smallpox	Children	,,
9				,,	,,	Triple	Triple	over	Smallpox
10				Smallpox		,,	,,	8 months	Triple
11						,,	,,	in	,,
12								following	,,
13								order	
	3rd Polio and 4th Triple } At 15–18 months				3rd Polio 7 months after 2nd. 4th Triple 12 months after 3rd.				3rd Polio 7 mths after 2nd
	Diphtheria + tetanus at school entry.								

Charles Wilcocks

GRECH, J. L. & CACHIA, E. A. **Acute Haemolytic Anaemia associated with Sulphadimidine Therapy.** [Memoranda.] *Brit. Med. J.* 1959, Dec. 12, 1309–10.

HOSPITAL AND OTHER CROSS INFECTIONS

BEEZY, R., BORMES, R. E. & KOCH, Marie L. **The Patient as a Source of Nosocomial Staphylococcal Infections.** *Antibiotic Med.* New York. 1959, July, v. 6, No. 7, 401–6. [14 refs.]

On one day clinical and bacteriological examinations were made of 937 patients [presumably all those present] in one hospital; 188 (20%) had clinical or subclinical infection, in 88 (9·4%) cases with coagulase-positive staphylococci. The rates for staphylococcal infection were 4·4, 10, and 18% respectively in the medical, surgical and geriatric services. 103 of the 207 separate infections were at a "foreign body site". 97% of the staphylococci were resistant to 1 or more antibiotics, and 73% to 3 or more. 60% of the staphylococci were typable by phage, with type 80/81 predominant. [There is no indication of the proportion of the infections that were associated with disease.]

R. E. O. Williams

SÁNCHEZ-TORRES, L. E., BAROCIO-LOZANO, L., BOLAÑOS-CASTELLANOS, G., BRÜGGEMANN-SCHMIDT, C. & VELASCO-HERNÁNDEZ, N. Epidemiologia de las infecciones estafilococicas. II. Estudio de los portadores de *Staphylococcus aureus* y sus diseminaciones en un servicio materno-infantil. [**Epidemiology of Staphylococcal Infections. II. Study of Carriers of *Staphylococcus aureus* in a Maternity Hospital**] *Rev. Latinoamer. Microbiol.* Mexico. 1959, June, v. 2, No. 2, 63–75, 2 figs. [32 refs.]

As a result of an epidemic of skin infections in a maternity hospital in Mexico a study was made of the carriage of *Staphylococcus aureus* among the staff and patients. 90% of the babies were found to have staphylococci by the 3rd day of life; the carrier rate for their mothers was 51%. Of 345 strains of staphylococci only 5% were sensitive to penicillin but 76% were sensitive to tetracycline and 80% to erythromycin. The commonest phage type was 77 (29% of all strains) with other group III strains and also 3B/3C making up the bulk of the remainder. [No type 80 strains are reported, and rather few Group I strains generally.] 24% of strains were untypeable. *R. E. O. Williams*

MITCHELL, A. A. B., TIMBURY, Morag C., PETTIGREW, J. B. & HUTCHISON, J. G. P. **An Epidemic of Postoperative Wound Infection by Type-80 Staphylococci.** *Lancet.* 1959, Oct. 3, 503–5, 1 fig.

A sudden increase in post-operative sepsis in the patients of 1 surgeon was found to be due to the fact that the surgeon had been operating while suffering from axillary boils; the wound staphylococci and those from the surgeon's lesion were phage-type 80. It is probable that an earlier set of infections had been attributable to another run of boils in the same surgeon, but although he had presumably carried the staphylococcus in the interval he seemed only to give rise to sepsis when he himself had a septic lesion.

A widespread epidemic of wound sepsis, with a total of 45 wounds infected, developed secondarily to the cases infected by the septic surgeon. These later infections were often deep-seated and appeared to result from theatre infection, yet they occurred after operations by all of 6 surgeons and no source was demonstrable within the theatre. It seems likely that the patients were bringing the ward staphylococci to the theatre with them. A possible source of infection was found when it was discovered that clean linen was returned to the wards, and used for the patients in the theatre, in the basket also used for soiled linen.

R. E. O. Williams

STENDERUP, A., BACH, Annelise, PEDERSEN, G. T. & ROSENDAL, Kirsten. **A Staphylococcal Epidemic in a Maternity Hospital.** *Acta Path. et Microb. Scandinavica.* 1959, v. 45, No. 1, 95–106, 1 fig. [14 refs.]

During the first 7 months of 1957 a maternity hospital in Jutland suffered an epidemic of infection among babies and mothers due to staphylococci of phage type 80. The infection rate was particularly high among the premature infants, who were kept in a special nursery and were often in incubators. Of 232 members of the staff of this hospital, 125 were carrying staphylococci and 20 had type 80. Because the typing results were not immediately available, all the carriers were treated 4 times daily by application of neomycin-bacitracin and tyrothricin ointments, the two being used on alternate 2-day periods for 8 days. Of 36 carriers tested 1 week after treatment 19 were free from staphylococci. 9 of 20 carriers of type 80 were, however, still positive.

Investigations were also made of the general hygiene of the wards. Type 80 staphylococci were found in many places and frequent cleaning was prescribed. The nurses' routines were also revised. There was a rapid decrease in the incidence of infection.

All of 38 type 80 strains were resistant to penicillin, but 27 were sensitive to tetracycline.

R. E. O. Williams

GILLESPIE, W. A., ALDER, V. G., AYLIFFE, G. A. J., BRADBEER, J. W. & WYPKEMA, W. **Staphylococcal Cross-Infection in Surgery. Effects of some Preventive Measures.** *Lancet.* 1959, Nov. 7, 781–4. [15 refs.]

This paper reports the incidence of staphylococcal infection of wounds in a surgical ward during a period of 2 years, during which various preventive measures were tried. In a control period of 6 months 1·6% of 2,356 surgical wounds suffered "major breakdown" due to infection apparently introduced at operation; in the same period 36% of the 592 drained or open wounds became colonized with staphylococci in the ward, "usually with little or no clinical effect". The serious complications arising from ward infections were pneumonia, urinary infections, etc. Most ward infections were caused by a few "epidemic" strains of staphylococci, probably transferred from patient to patient. The same types were found in about ¼ of the theatre infections. No example of infection from a nasal carrier in the nursing staff was discovered. Even with theatre infection, wounds needing drainage were infected more often (3·5%) than undrained, closed, wounds (1·0%).

The first additional precautions to be introduced were the local antibiotic (neomycin, gramicidin, bacitracin etc.) treatment of nurses and patients found to be nasal carriers of penicillin-resistant staphylococci, and formaldehyde disinfection of blankets [see GILLESPIE and ALDER, this *Bulletin*, 1957, v. 32, 720]. No effect on the rate of colonization of wounds by staphylococci could be detected though there was some suggestion that the incidence of theatre infection was reduced.

The next round of precautions included disinfection of crockery and the barber's shaving brushes etc., use of chlorohexidine hand cream by the nurses, and disinfection of baths. No effect on wound colonization was seen. Subsequently a "Polybactrin" (neomycin, bacitracin, and polymyxin) spray was used on open wounds in the wards to prevent colonization and to disinfect the dressings; a slight but statistically insignificant reduction in colonization was seen.

The final precautions included the use of vacuum cleaners in the wards, paper towels, hexachlorophene soap for nurses and patients, hexachlorophene disinfection of baths [AYLIFFE *et al.*, *Lancet*, 1959, v. 2, 456], and, lastly, the twice-daily application of neomycin-bacitracin ointment to the anterior nares of all the patients all the time that they were in the ward.

With this regimen, the incidence of colonization with penicillin-resistant staphylococci was 8% of 108 open wounds compared with 42% of 95 in the previous year. Colonization with penicillin-sensitive staphylococci was unchanged at about 4–5%. There was also a reduced incidence of pneumonia etc.

Improvements in the operating theatre ventilation had no obvious effect on infections in patients from control wards, but the hygienic improvements in the test ward seemed to be followed by a reduction in theatre infections in patients from that ward.

R. E. O. Williams

See also p. 283, VOGELSANG & HAALAND, **Studies of Pathogenic Staphylococci in the Upper Respiratory Tract of Members of Hospital Staff. 1. Carrier Rate of Pathogenic Staphylococci. 2. Antibiogram Determinations. 3. Bacteriophage Typing. 4. Phage Groups and Phage Patterns of Antibiotic Resistant Staphylococcal Strains.**

STERN, H., ELEK, S. D., MILLAR, D. M. & ANDERSON, H. F. **Herpetic Whitlow. A Form of Cross-Infection in Hospitals.** *Lancet.* 1959, Nov. 21, 871–4, 3 figs. [11 refs.]

54 cases of septic lesions occurring on the fingers of nurses working in a neurosurgical unit were noted. Each lesion started as an area of irritation developing into a vesicle, which coalesced with other vesicles, commonly under the nail, and which was very painful. No pus formed. After 10 days, the pain suddenly disappeared and the vesicles dried up. The lesions resisted all forms of treatment. They sometimes recurred at the original site, from 1 month to 3 years later. There was no history of trauma and no constitutional upset. On culture, fluid from the vesicles was either sterile or contained organisms which were contaminants. (The lesion could be mistaken for a staphylococcal one when it became infected, secondarily, with this organism, which was always of the same phage type as the staphylococci in the nurse's nose.) When vesicular fluid was inoculated into human amnion tissue cultures it produced changes characteristic

of herpes simplex virus, and the presence of the virus was confirmed by neutralization tests with immune sera. A rise in neutralizing antibody in the nurses' serum was also demonstrated, and all but 2 of the cases from which virus was isolated appeared to be primary infections, as no antibody was present before the lesion occurred. 49% of the nurses were found to be susceptible. There was no serological evidence of sub-clinical infection.

Only 1·2% of patients in the neurosurgical unit were salivary carriers of herpes, and they could not account for all the infections. However, virus was isolated from the bronchial secretions of 4 of 63 (6·5%) patients who had undergone cranial surgery and who had no clinical signs of herpes. It seemed that the nurses were infected when, with bare hands, they attached catheters to tracheotomy tubes in these patients, in order to remove bronchial secretions. Once the source of virus was recognized the wearing of rubber gloves proved to be sufficient protection against infection.

Janice Taverne

CALNAN, J. S. **Clean Blankets. New Boilable Bedcover.** *Lancet.* 1959, Feb. 7, 300–302, 1 fig.

The importance of blankets as a reservoir of bacterial contamination in hospitals is now widely accepted. One method of reducing this risk is to devise methods of laundering conventional woollen blankets which will disinfect them without causing undue damage [see BLOWERS and WALLACE, this *Bulletin*, 1955, v. 30, 897]. FRISBY [*Ibid.*, 1958, v. 33, 63] suggested that another and possibly better solution was to devise blankets which would withstand repeated boiling. The present paper describes such a blanket, consisting of a central layer of cellular meshed material made of viscose staple and cotton, quilted between two sheets of cotton. The blankets were used for over a year in a 4-bed ward at a plastic surgery unit, with wool blankets in a similar ward as a control. The cellular blankets were changed weekly (and presumably boiled). The new blankets were acceptable to patients and nurses. 2 per bed were used as equivalent to 3 woollen blankets, and were adequate in an average ward temperature of 65°F. (18°C.). Objective comparisons showed that the new blanket cost 2½ times as much as a single-bed sheet, while the old cost 3–4 times as much. The weight was 22oz. per sq. yd. for the new and 15 for the old. Wool blankets had an estimated life of 9 years, with no washing, cellular ones 3¾ years (? 150 washings) as for a sheet. Wool blankets shrank 20% or more when washed, cellular blankets 5–6%. The new blankets had longer fibres, leading to a noticeable reduction in dust and fluff.

The bacteriological aspects of the trial will be described in a forthcoming paper by Frisby. The incidence of wound infection was a little lower in patients using the new blankets, but as the overall rate was very low the difference was not significant.

J. C. Kelsey

COCCAL INFECTIONS

The order in this section is:—*Staphylococcus, Streptococcus, Neisseria, Others.*

MARKHAM, N. P. & STENHOUSE, A. C. **A Bacteriological Investigation of Wound Infections in Rarotonga, Cook Islands.** *Trans. Roy. Soc. Trop. Med. & Hyg.* 1959, Sept., v. 53, No. 5, 404–9. [11 refs.]

Bacteriological studies were made of 195 skin lesions seen in Rarotonga, Cook Islands; 22 were in Europeans and 173 in Rarotongans and most were impetiginous. Over 70% of the lesions yielded coagulase positive staphylococci but whereas, of the Europeans' lesions, 46% yielded β-haemolytic streptococci and none gave *Corynebacterium diphtheriae,* for the Rarotongans the proportions were 70% and 32%. 56% (9) of the Europeans' staphylococci were penicillin-resistant compared with 19% (26) of the Rarotongans'.

Among healthy persons, 17·5% of 80 yielded β-haemolytic streptococci from the throat and 13% of 48 yielded streptococci from the skin. Coagulase-positive staphylococci and haemolytic streptococci were isolated from flies that had been feeding on exposed sores. [The source of the patients—whether in hospital or not—is not explicitly stated, nor the method of selection of the healthy individuals.] *R. E. O. Williams*

BERNHARD, G. C. & STOLLERMAN, G. H. **Serum Inhibition of Streptococcal Diphosphopyridine Nucleotidase in Uncomplicated Streptococcal Pharyngitis and in Rheumatic Fever.** *J. Clin. Investigation.* 1959, Nov., v. 38, No. 11, 1942–9, 1 fig. [18 refs.]

CRUICKSHANK, R. & GLYNN, A. A. **Rheumatic Fever. Epidemiology and Prevention. The Proceedings of a Seminar held at the International Children's Centre, Paris, 25–27 September 1956.**

This book is reviewed on p. 327.

BERKSON, D. M., PERLMAN, L. & MILLER, A. J. **Fulminating Meningococcemia (Waterhouse-Friderichsen Syndrome). Report of Two Patients successfully treated with Chemotherapy, Vasopressors, and Adrenal Steroids.** *J. Amer. Med. Ass.* 1959, July 18, v. 170, No. 12, 1387–90. [15 refs.]

A woman of 59 developed the ecchymotic rash and shock symptoms of this syndrome (but with measurable blood pressure) on the third day of the disease, by which time the spinal fluid was cloudy with numerous polynuclear cells. She had vigorous antimicrobial treatment (a sulphonamide, penicillin and chloramphenicol) as well as steroid therapy and continuous non-adrenaline drip over a period of 4 days, after which sulphonamide alone was continued for some 2 weeks. Recovery was uneventful

A man of 40 had acute meningococcaemia with petechial rash, and rapid development of suppurative meningitis; his blood pressure fell as low as 70/40 mm. Hg. These are the signs of meningococcal meningitis with a suspicion of a commencing Waterhouse-Friderichsen syndrome. Under similar combined therapy his recovery was rapid.

[There is nothing remarkable about the recovery of these 2 patients. Neither was an extreme case and the age-group was much more favourable for prognosis than that of children. The weight of evidence is considered to be in favour of adrenal replacement therapy, although adrenal haemorrhage is not always present. The references given are exclusively American.]

H. Stanley Banks

BACTERIAL INFECTIONS

The order in this section is:—*General, Corynebacterium, Haemophilus, Enteric bacteria, Brucella, Pasteurella, Bacillus, Anaerobes, Spirochaetes, Others.*

EISELEN, H. H., ROBINSON, Mary & MASON, J. H. **Immunization of the Rural Bantu against Diphtheria in the Northern Transvaal. I. Immunity produced after One, and after Two, Injections of Alum-Precipitated Toxoid.** *South African Med. J.* 1959, Oct. 17, v. 33, No. 42, 881–4, 2 figs.

For the past 10 years a sporadic incidence of diphtheria has existed among the Bantu of Northern Transvaal, especially in the young children. A normal immunization programme could not be carried out and it was decided to study the immunizing effect of 1 and of 2 injections of alum-precipitated toxoid (APT). In a rural location containing 12,000 to 15,000 Bantu subcutaneous doses of 0·5 ml. of APT, 50 Lf per ml. were given to 262 babies aged 6 to 24 months, whose serum contained less than 0·001 unit of antitoxin per ml. Group 1 received 2 doses of APT at 6 to 8 weeks' interval, Group 2 received 1 dose, Group 3 served as a control. Sera from all the children of the groups were tested for antitoxin at 3, 6, 12 and 24 months after the start of the test. A fourth group consisted of children whose sera contained above 0·001 unit of antitoxin per ml.; these received 1 dose of APT to boost immunity and their sera were examined 3 and 12 months later for comparison with Group 2. In addition 3,825 children received 1 dose of APT and of these 3,084 received a second dose 8 weeks later, in order to control natural transmission of diphtheria which might affect the experiment.

The tests showed that 2 doses produced better immunity than 1. All children receiving 1 dose showed antitoxin at 3 and 6 months but at 2 years 14% were Schick-negative (*i.e.*, under 0·004 unit antitoxin per ml.). The existence of subclinical transmission was shown by the development of antitoxin in some controls. In Group 2, 38·7% were protected at 12 months as compared with 91% of Group 4 and 88·2% of Group 1. It was considered that while 1 dose of APT was not to be recommended as a substitute for the 2- or 3-dose method of immunization, it had a definite value where campaigns were difficult, producing immunity even if transient and boosting immunity in those who had previously suffered silent infections.

T. H. Davey

MASON, J. H., ROBINSON, Mary & EISELEN, H. H. **Immunization of the Rural Bantu against Diphtheria in the Northern Transvaal. II. Active Immunization of Babies possessing Maternally-Transmitted Antitoxin.** *South African Med. J.* 1959, Oct. 31, v. 33, No. 44, 920–21.

"The opportunity occurred of carrying out an experiment in very young Bantu babies in the Pietersburg District of the Northern Transvaal" where "inapparent infections" of diphtheria are common [see Part I above]. It has been shown that in European babies maternally transmitted antitoxin interferes with the production of immunity against diphtheria if the serum of the baby contains more than 0·04 unit of antitoxin per ml. at the time of immunization [*ibid.*, 1954, v. 29, 574, 1021].

In the investigation described here blood samples were taken from 125 Bantu babies between the ages of 60 and 129 days. Serum-antitoxin values were obtained from the blood samples of 100 of these children who were given a first inoculation of alum-precipitated toxoid immediately after bleeding and a second dose 6–8 weeks later. Further blood samples were taken and examined at 3, 6, 12 and 24 months after the first injection of APT. The results of the investigation which are described and set out in tables indicate that in these Bantu babies "active immunity against diphtheria is depressed when the circulating antitoxin titre is 0·1 unit or more per ml. of serum." It was found that the sera of 21% of young babies contained this amount of antitoxin. The opinion is expressed that although ideally immunization should not be carried out in babies until they have reached the age of 4 months or more, when antitoxin level has fallen to 0·04 unit or less, in the Northern Transvaal "the practical approach is probably the active immunization of every child who presents himself" because only one-fifth of the babies have high titres and some of these respond satisfactorily.

M. E. Delafield

VINCIGUERRA, M. Sulla bonifica dei portatori di *B. difterico* con tirotricina. [**The Treatment of *Corynebacterium diphtheriae* Carriers with Tyrothricin**] *Riv. Italiana d'Igiene.* 1959, May–June, v. 19, Nos. 5/6, 166–72. [13 refs.] English summary (4 lines).

The author first gives a list of antibiotics active against *C. diphtheriae* and mentions that tyrothricin is made up of 2 components, gramicidin and tyrocidine; the compound is insoluble in water but soluble in alcohol, glycerol, and the glycols. It is stable at 100°C. both in the dry state and in glycol solution.

He carried out trials with this antibiotic in the city of Matera (Lucania) where he is the Medical Officer of Health, on 1,751 children aged 6–12 years, some of whom were carriers, dividing them into 3 classes: (a)

51 who had had diphtheria; (b) 317 contacts; (c) 1,383 who had neither disease nor contact. Of these 3 groups, 26, 30 and 58 children respectively were positive for *C. diphtheriae*. These 114 (6·5%) were then treated as follows. The pharynx was cleaned and the antibiotic, dissolved in a polyethylene glycol solution, was sprayed by a nebulizer on to the oropharynx and instilled into the nose. Swabs were taken the next day and 15, 30 and 45 days later. The results were that 93% were negative after the first application and 100% after the second.

The author considers this method effective, and much better and less costly than if other antibiotics are used.

W. K. Dunscombe

VARELA, G., KEAN, B. H., BARRETT, Elizabeth L. & KEEGAN, Caroline J. **The Diarrhea of Travelers. II. Bacteriologic Studies of U.S. Students in Mexico.** *Amer. J. Trop. Med. & Hyg.* 1959, May, v. 8, No. 3, 353–7.

This paper is the second publication on the diarrhoea of travellers [see this *Bulletin*, 1959, v. 34, 206 and below] and in it there is a report of bacteriological studies on 62 students from the United States recently arrived in Mexico. These students—23 male and 39 female—were asked to provide stool specimens on 14 consecutive days. They had been in Mexico from 1 to 11 days before providing the first specimen.

The methods and details of examination of the stool specimens on toilet paper are described [see also this *Bulletin*, 1956, v. 31, 664]. Bacteria isolated which were, *for purposes of classification only*, considered as pathogenic or possibly pathogenic are listed: "(1) *Salmonella* sp. (2) *Shigella* sp. (3) *Klebsiella* sp. (4) *Paracolobactrum* sp. (5) *Proteus* sp. (6) *Escherichia coli* O26, O44, O55, O86, O111, O112, O119, O124, O125, O126, O127, O128".

The results of the study are described with tables of particulars. 27 of the 62 students became ill with diarrhoea of varying severity during the 14 days of study.

It is noteworthy that "*Salmonella* and *Shigella* were not cultured in any case". Although the other organisms on the list of pathogens or possible pathogens were found somewhat more frequently in students with diarrhoea than in those who remained well yet "a definite causal relationship was not established".

M. E. Delafield

KEAN, B. H. & WATERS, S. R. **The Diarrhea of Travelers. III. Drug Prophylaxis in Mexico.** *New England J. of Med.* 1959, July 9, v. 261, No. 2, 71–4.

Several papers have appeared recently on the well-known diarrhoea of travellers [see also this *Bulletin*, 1959, v. 34, 206), which is known under a variety of names such as "Delhi belly", "Turkey trot", "Gippy tummy", "Montezuma's revenge" and the "G.I.'s".

As "considerable orthodox medical support for the prophylactic use of drugs is available" a study on this point was made and is reported in the present paper. Mexico was chosen as the site of the experiment because of the large number of American tourists (over 500,000 annually), the frequency among these visitors of diarrhoea (known locally as "turista") and the excellent laboratory facilities "which permitted concurrent bacteriologic studies reported elsewhere". In preliminary work it was found that in about one-third of 1,265 visitors spending 7 days or more in Mexico diarrhoea developed. Of 444 of these visitors who had taken medicine of various kinds to prevent diarrhoea 236 (53%) became ill. Of 821 who did not take drugs, only 126 (15%) contracted diarrhoea. "These figures were interpreted with caution since some of those who took drugs may have started treatment just *after* the onset of early symptoms."

A controlled experiment was then made on 610 American students new to Mexico arriving to study at the University or City College. They were instructed to take 1 tablet night and morning for 14 days of 1 of the 3 drugs chosen for testing. The identity of the tablets was unknown to both students and investigators. The tablets contained either (1) 375 mgm. of iodochlor-hydroxyquinone—a substance used widely by tourists often on recommendation by physicians, or (2) 375 mgm. of neomycin sulphate with 120 mgm. of kaolin and 5 mgm. of pectin—on the theory that bacterial enteropathogens might be responsible for the diarrhoea or (3) 375 mgm. of lactose as a placebo. The drugs were started 1–8 days after arrival in Mexico.

The results obtained with the 3 groups of about 200 students each were that diarrhoea occurred in 38·6% of those taking iodochlor-hydroxyquinone, 20·1% of the neomycin group and 33·6% of those taking the lactose. Unfortunately the neomycin tablets contained also small amounts of kaolin and pectin. Further evaluation of neomycin and other prophylactic preparations is necessary.

M. E. Delafield

TESCH, J. W. & SCHÖNFELD, J. K. Een epidemie, verwekt door *Shigella sonnei* in 1955. [**An Epidemic due to *Shigella sonnei* in 1955**] *Nederl. Tijdschr. v. Geneesk.* 1959, June 27, v. 103, No. 26, 1333–8, 2 figs. –[20 refs.] English summary.

An outbreak of *Shigella sonnei* infection occurred in Rotterdam in 1955; 322 cases were notified, of which 65 occurred in a children's home. The illness was mild and there were no fatal cases. Among 537 members of 114 affected families the disease mostly occurred in children below the age of 14 years. The results of bacteriological examinations are presented in a number of tables.

D. J. Bauer

HAVLÍK, J., KOTT, B. & POTUZNÍK, V. **The Indirect Haemagglutination Test in Dysentery caused by *Shigella sonnei* and *Shigella flexneri*.** *J. Clin. Path.* 1959, Sept., v. 12, No. 5, 440–43. [12 refs.]

2 polyvalent antigens were used, one comprising *Shigella sonnei* S and R and the other *Sh. flexneri* 1–6. Human O Rh-negative red cells were sensitized with these antigens and first tested against a number of control sera containing antibodies against salmonella, coli and proteus antigens for preliminary checks on specificity. These were satisfactory and then sera from

dysentery patients from whom either Flexner or Sonne bacilli had previously been isolated were examined. Compared with the bacterial agglutination test the titres obtained in the haemagglutination test were always much higher—often as much as 10 times. The results were clear-cut when the sensitizing antigen was the homologous type but when sera were examined by parallel tests with two lots of red cells, one sensitized with Flexner-antigens and the other with Sonne-antigens some cross reactions were noted. Examination of further specimens of sera obtained at intervals prevented misinterpretation and indicated that the cross-reactions were associated with previous infections with one or the other types—particularly with *Sh. sonnei*.

The bacterial agglutination test on dysenteric patients' sera has never been regarded as a very valuable diagnostic test when carried out at some time distant to the acute clinical infection because so many persons without known histories of bacillary dysentery show low serum titres of agglutinins to *Sh. flexneri*. Whether the haemagglutination test is likely to prove more valuable remains to be seen. It will be recalled that NETER and WALKER [this *Bulletin*, 1955, v. 30, 576], who introduced the test for bacillary dysentery, claimed that specific haemagglutinins were to be found during the acute stage of the disease. *H. J. Bensted*

VIARRE, M., GAUDIER, B. & BUTTIAUX, R. Remarques épidémiologiques et bactériologiques sur une épidémie de gastro-entérites infantiles associées à *Escherichia coli* O127:B8. [**Epidemiological and Bacteriological Considerations on an Infantile Gastro-Epidemic from which *E. coli* O127 was isolated**] *Ann. Inst. Pasteur.* 1959, Sept., v. 97, No. 3, 424–8.

Until the second half of 1958 *Escherichia coli* O111 was the predominant organism associated with outbreaks of infantile gastro-enteritis in the area in France served by the authors. Up to that time O127 had rarely been isolated but from July until the end of the year isolations became increasingly common; in the last quarter (Oct.–Dec.) the O127 cases were more than 4 times the number of the O111 cases. The detail of the change is shown in the accompanying table:

E. coli sero-type	Years 1958 1st quarter	2nd quarter	3rd quarter	4th quarter
O111	98	140	92	24
O127	3	4	55	111
others	13	5	12	20

Clinically the severity of the outbreak is recorded as being of the same order as that of the O111 cases; the mortality was about 2%. The tetracyclines were not very effective. All the strains examined were sensitive to "furadoïne" and colimycin: half were sensitive to neomycin. Colimycin proved to be very successful and the majority of the patients responded well and were clear in 3–7 days.

[Most workers interested in gastro-enteritis in infants have recorded changes in the incidence of the various O-types of *E. coli* associated with infantile gastro-enteritis over the years and O127 has been reported from several different geographical areas in recent times. In this connexion, however, it is the integrated reports of the Public Health Laboratory Service of Britain that have particular value in epidemiological studies where local reports may be heavily weighted by epidemic data and are not necessarily supported by nation-wide reports. Be this as it may, this report is of considerable interest.] *H. J. Bensted*

AWAD, N. A. **Urinary 17-Ketosteroid Excretion, Blood Eosinophil Level and Adrenocortical Function in Human Brucellosis.** *Trans. Roy. Soc. Trop. Med. & Hyg.* 1959, July, v. 53, No. 4, 349–61, 3 figs. [39 refs.]

This investigation was undertaken to elucidate the adrenocortical function in human brucellosis as it has been observed that the administration of cortisone or corticotrophin (ACTH) in acute brucellosis appears to enhance the action of antibiotics.

The urinary total neutral 17-ketosteroid output and blood eosinophil levels and their alteration after the administration of ACTH were studied in adult male Egyptians suffering from brucellosis before and at various stages after its treatment.

In patients with brucellosis the mean output of 17-ketosteroids was low and was thought to be related to affected liver function. It and the blood eosinophil level were restored to normal after cure. The response to intramuscular ACTH was normal in extent but was delayed in onset and more prolonged than in normal subjects. Similar changes were found with repeated intravenous ACTH infusions. In addition, in the acute stage of the disease the increase in the urinary 17-ketosteroid excretion was not sustained and fell while the stimulus of the hormone was still maintained. After antibiotic treatment this abnormal reaction disappeared. It is concluded that in acute brucellosis the adrenal cortex becomes at first hypersensitive to the ACTH stimulus but later becomes exhausted and insensitive to further stimulation.

[A painstaking piece of work. While it supports the efficacy of ACTH in acute brucellosis, the complex response of the adrenals indicates that the clinical use of ACTH should be limited to the rare example where antibiotics alone may be inadequate to control acute manifestations.] *Frederick J. Wright*

AWAD, N. A. **Experimental Brucellosis and the Adrenals. Effects of Ascorbic Acid and A.C.T.H.** *J. Trop. Med. & Hyg.* 1959, Sept., v. 62, No. 9, 216–22, 2 figs. [25 refs.]

The author describes observations made on adrenal function in rats and guineapigs experimentally infected with *Brucella abortus*. All results were compared with observations made on uninfected animals used as controls. The adrenal functions were deduced from changes in weight, ascorbic acid and cholesterol content before and after infection and after the administration of ascorbic acid or corticotrophin (ACTH).

Observations were made at 3–4, 7–8 and 11–12 weeks after infection. Guineapigs, like man, cannot synthesize ascorbic acid but rats differ in this respect, also they are relatively resistant to *Br. abortus* and the relative content of the various steroids in the adrenals differs. By using both animals it was hoped to throw light on adrenal responses.

In untreated guineapigs, 3–4 weeks after inoculation with *Br. abortus*, the adrenal gland was slightly enlarged, the ascorbic acid content lower and the cholesterol content slightly higher than in normal controls, indicating a stimulation of pituitary function with over-production of endogenous corticotrophin. This overactivity of the pituitary continued at 7–8 weeks after infection but a low cholesterol content suggested increased production of adrenocortical hormones. At 11–12 weeks the return of adrenal ascorbic acid to normal suggested adaptation to the stress of the disease. In untreated rats at 3–4 weeks there was evidence of depression of pituitary function and decreased production of corticotrophin. These changes continued at 7–8 weeks but at 11–12 weeks the depression of the pituitary was removed indicating favourable adaptation.

Parenteral ascorbic acid in large doses led to hyperactivity of the pituitary in normal guineapigs and an increased adrenal response in infected animals. But in normal rats there was a marked decrease in weight of the adrenals, indicating increased hormone production and in infected animals much less decrease in weight.

ACTH administered to guineapigs with brucellosis tended to reverse the changes caused in adrenal function by the infection. Higher doses of ACTH were required to produce a similar effect in rats suffering from brucellosis.

[These experiments indicate that multiple indices of adrenal function and a variety of experimental animals should be studied before applying the results to the treatment of human infections.] *Frederick J. Wright*

ZINNEMAN, H. H., GLENCHUR, H. & HALL, W. H. **The Nature of Blocking Antibodies in Human Brucellosis.** *J. Immunology.* 1959, Aug., v. 83, No. 2, 206–12, 8 figs. [20 refs.]

In a study of 8 cases of human brucellosis, it was found that the agglutinating antibody appeared at an early stage in the course of the disease but had virtually disappeared in chronic infections, whereas blocking antibody first appeared at a later stage, persisted longer, and was the predominant antibody of chronic infections.

When the sera were fractionated by starch-gel electrophoresis it was shown that the agglutinating antibody was distributed between the gamma and beta globulins, whereas in chronic infections the blocking antibody was restricted to the beta globulins. In one case which was followed from an early stage of the disease the blocking antibody was first detected in the gamma globulin but later was restricted to the beta globulin.

It is suggested that the prozone phenomenon so often seen in *Brucella* agglutination titrations is due not to an excess of agglutinating antibody but rather to the simultaneous presence of blocking antibody.

J. Swanson Beck

ALLISON, F., Jr. **Pleuropulmonary Tularemia.** *Southern Med. J.* 1959, Sept., v. 52, No. 9, 1019–26, 3 figs. [20 refs.] Discussion 1026–7.

The author cites experiences from 10 cases of tularaemia recognized in the wards of the University Hospital and Jackson Veterans' Hospital, Jackson, Mississippi between July 1955 and July 1958. 3 case histories are recorded in detail. 7 of the 10 exhibited pulmonary involvement, this high proportion reflecting the severity of the cases which required admission to hospital. The author emphasizes that now that the danger of acquiring the infection from wild rabbits is well-known, a relatively greater incidence of tick-borne infections is being found. This is probably because such infections are more difficult to prevent.

When a local skin lesion is present at the portal of entry, the diagnosis is easier than in cryptic infections, but even if an ulcer is present it may pass unrecognized if mental confusion and other signs of serious illness overshadow the picture. In one such patient there was urinary retention due to an enlarged prostate gland and a high level of azotaemia which persisted after adequate urinary drainage. The azotaemia proved to be due to tularaemia, a feature of some severe cases as noted by GIDDENS *et al.* (*J. Louisiana Med. Soc.*, 1957, v. 109, 93). Toxic jaundice probably attributable to cloudy swelling of hepatic cells occurred in one patient. Pleural effusions are a common accompaniment of pulmonary tularaemia and *Brucella tularensis* can sometimes be cultured from the pleural fluid. When the organism has not been recovered from the blood or elsewhere, confirmation of the diagnosis rests on observing a marked rise of agglutination titre which may also be accompanied by a moderate titre of agglutination of a suspension of *Brucella abortus*. This is apparently due to a common protein antigen.

The drug of choice in treatment is streptomycin. In discussion, H. E. DASCOMB suggested that if treatment were begun early, then it should be more prolonged, as cure depends on an adequate antibody response which probably takes about 18 days to develop.

Frederick J. Wright

MINISTRY OF LABOUR. **Report of the Committee of Inquiry on Anthrax** [LEVY, R. F., Chairman]. Cmnd. 846. pp. viii + 222 + 4 loose-leaf. 1959. London: H.M. Stationery Office. [12s.]

The terms of reference of the Committee which here presents its report were "to be a Committee to consider the existing legal provisions concerning the importation of goods infected or likely to infected with anthrax and the precautions to be taken in connection with such imported goods for the protection of the health of persons and to make recommendations".

The Report is extremely well presented and contains a vast amount of information and many recommendations. The Committee held 20 meetings; interviewed 71 witnesses representing government departments, trade unions, trade organizations, companies and societies; received many written statements and visited a number of factories.

The first chapter contains some account of anthrax infections with statistics of incidence such as that the

number of cases of external anthrax notified to the Chief Inspector of Factories in 1900–1904 was 208 with 44 deaths reduced by 1955–58 to 53 cases with 3 deaths. Succeeding chapters deal with anthrax due to wool and hair; horsehair, bristles, hog and cows hair, etc.; hides and skins; bones and bone meal and Japanese shaving brushes. The arrangements for early diagnosis and treatment of suspected cases of anthrax as well as precautions of general application in all factories where anthrax-bearing materials are handled are considered in separate chapters. Finally there is mention of the possibility of immunization against anthrax and the possibility of disinfecting anthrax-bearing materials by irradiation. More than half of the Report is given up to the Memorandum of Evidence submitted by the Ministry of Labour and National Service containing 14 appendices on matters such as present legal provisions, history of Government action, statistical information and detailed analyses of recorded cases of anthrax in industry.

There is a good summary of the detailed recommendations which are made in the separate chapters of the Report. *M. E. Delafield*

GRÜNBAUM, B. & HERTWIG, F. Über das Vorkommen von Tetanusbazillen im Strassenstaub von Berlin in den Jahren 1957/58. **[Tetanus Bacilli in the Dust of Berlin Streets in 1957–58]** *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1959, Sept.–Oct., v. 5, No. 5, 303–7.

The work here described was undertaken to assess the magnitude of the risk of tetanus among city dwellers of Berlin. Some of the literature on the incidence of tetanus in Germany shows that this risk is by no means negligible.

In 1957–58, 400 samples of street dust were taken from different districts of Berlin and were examined by methods which are described for the presence of tetanus bacilli. The 3 methods used were: (1) inoculation on glucose blood agar plates, (2) inoculation of Tarozzi broth, and (3) direct animal inoculation. The average results obtained were 5% positives for tetanus with the plate method, 29·7% positive on broth culture and 55% positive on direct inoculation into white mice.

These results indicate the need for much greater use of active immunization in Berlin. *M. E. Delafield*

PATEL, J. C. & JOAG, G. G. **Grading of Tetanus to Evaluate Prognosis.** *Indian J. Med. Sci.* 1959, Oct., v. 13, No. 10, 834–40. [11 refs.]

From an analysis of 1,398 cases of tetanus in Bombay the authors graded the disease as regards prognosis into 5 groups based on 5 symptoms or signs. These were lockjaw, spasms, incubation period less than 7 days, period of onset less than 48 hours, and temperature of 99°F. within 24 hours of admission to hospital. In patients in group 5, *i.e.* having all 5 symptoms the mortality was 83·5%, in group 1, *i.e.* having only 1 symptom, it was nil. *A. B. Christie*

POULSEN, P. A. & NORBY, G. **Tetanus. An Analysis of 40 Cases treated during the Period 1939–1959.** *Danish Med. Bull.* 1959, Oct., v. 6, No. 7, 223–7, 1 fig. [10 refs.]

This is a review of 40 patients in Denmark suffering from tetanus, 22 treated in the period 1939–1953 and 18 in the period 1955–1959. In the earlier period treatment was by simple sedation only; in the latter treatment as for respiratory poliomyelitis was given. Tracheotomy was done in 2 of the 22 patients in the earlier period, and in 13 of the 18 in the later period. The disease appeared to be more severe in the later period yet the mortality was 28% compared with 50% in the earlier period. Autopsy showed that pulmonary complications, especially atelectasis and bronchopneumonia were predominant in both groups. The authors feel that the application to tetanus of the principles used in the treatment of respiratory poliomyelitis marks a substantial advance. *A. B. Christie*

LEONARDI, G. & ZULIAN, G. Finalità e modalità pratiche del trattamento del tetano nella casistica giornaliera di una zona agricola. **[The Practical Aims and Methods of Treatment of Tetanus in the daily Practice of an Agricultural Zone]** *Giorn. di Malattie Infettive e Parassit.* 1959, Aug., v. 11, No. 8, 710–14. [21 refs.]

Working in an agricultural zone, where the incidence of tetanus was 4 times the national average for Italy, the authors had only 6 cases of tetanus in hospital during 1958 and at no time was there more than 1 patient with tetanus in hospital. They argue therefore that specialized centres are not justified in such an area and claim moreover that transport of a patient over long distances leads to delay in treatment and exposes patients to excessive harmful stimuli. 2 of their 6 patients died. Treatment was by tetanus antitoxin in doses of about 500,000 international units over a period of 5 days followed by a course of active immunization; antibiotics and Mephenesin alternating with a combination of chlorpromazine, promethazine and pethidine were also given. The length of incubation period and invasion period and the use of prophylactic antitoxin had no effect on the prognosis. The most important thing was the rapid administration of treatment. *A. B. Christie*

SHANKER, A. & MEHROTRA, L. S. **A Preliminary Report on Chlorpromazine in Tetanus.** *Brit. Med. J.* 1959, Nov. 28, 1150–52. [17 refs.]

Chlorpromazine was given to a group of 15 patients and the results compared with those in another group of 15 patients treated with paraldehyde. The dose of chlorpromazine was 50 to 100 mgm. every 12 hours and of paraldehyde 6 ml. every 4 or 6 hours. If spasms developed between the injections of chlorpromazine, the patients were given paraldehyde to control them. The authors feel that chlorpromazine is a much more successful drug than paraldehyde in the treatment of tetanus but do not claim that their figures are significant. *A. B. Christie*

HAMILTON, W. **Treatment of Tetanus with Chlorpromazine. Two Case-Reports.** *Brit. J. Clin. Practice.* 1959, Nov., v. 13, No. 11, 823–5.

2 patients suffering from tetanus were treated with chlorpromazine along with other sedatives. Given intravenously it was more efficient in one patient (who survived) than twice the dose given intramuscularly. Bed-making, toilet and feeding were done immediately after an injection. Sweating on the forehead came to be recognized as an indication for further chlorpromazine. *A. B. Christie*

LAWSON, E. A. & BANKOLE, M. A. **Continuous Intravenous Sedation in Severe Tetanus.** [Correspondence.] *Lancet.* 1959, Nov. 21, 917–18. [18 refs.]

A Nigerian patient, aged 29 suffering from tetanus was first treated with chlorpromazine and sodium amylobarbitone. When these failed to control spasms, a polyethylene cannula was introduced into the inferior vena cava and 2 litres of a solution of 2 gm. of sodium thiopentone in a litre of 4·3% glucose in 0·18% saline solution were given daily by continuous infusion for 34 days. The spasms were controlled. The patient was fed by intragastric drip and was turned every 2 hours, the mouth and pharynx being sucked out each time. He was managed throughout by nursing staff with only occasional attention from doctors. The authors regard this as a relatively simple method of treatment. *A. B. Christie*

PERLSTEIN, M. A. **Control of Tetanus Spasms by Administration of Meprobamate.** *J. Amer. Med. Ass.* 1959, Aug. 15, v. 170, No. 16, 1902–8, 2 figs. [14 refs.]

Given in intramuscular doses of 400 mgm. every 4 hours, meprobamate had a marked effect in reducing tetanic spasms in 39 patients. It reduced spasms somatically induced such as by pin-prick, bright light and noise but not those viscerally induced such as by bladder or intratracheal catheters. Patients remained quiet and relaxed even when the dose of pentobarbital was reduced to 3 grains instead of 18 to 24 grains per day. *A. B. Christie*

DOTTI, F. Ulteriori ricerche sulla diffusione della leptospirosi nella Valle Padana. (Casistica raccolta in provincia di Mantova). [**Further Observations on the Incidence of Leptospirosis in the Po Valley**] *Igiene Moderna.* 1958, Nov.–Dec., v. 51, Nos. 11/12, 867–82. [20 refs.] English summary.

The author studied the incidence of leptospirosis in the valley of the river Po in North Italy during the two 4-year periods 1947–50 and 1954–57. Although the absolute numbers of cases for which records were available were nearly equal for the 2 periods, the series were not really comparable. All the patients from 1947 to 1950 were treated in hospital whereas only 115 (52%) of the 1954–57 series required hospital treatment. If one may assume that a similar proportion of mild cases were treated at home (and not reported) in the first period, it would appear that there had been a marked fall in incidence.

Differences were noted also in the relative proportions of infections caused by various serotypes. In round numbers, cases due to *Leptospira icterohaemorrhagiae* fell from 66% to 43% while those caused by *L. pomona* rose from 20% to 37%. The figures for *L. bataviae* showed less change at 7% and 10%, but *L. hyos* rose from 0·5% to 8%.

Incidence was highest among swineherds and ricefield workers, and vaccination of these workers is recommended. *J. C. Broom*

RAMOS-MORALES, F., DÍAZ-RIVERA, R. S., CINTRÓN-RIVERA, A. A., RULLÁN, J. A., BENENSON, A. S. & ACOSTA-MATIENZO, Josefina. **The Pathogenesis of Leptospiral Jaundice.** *Ann. Intern. Med.* 1959, Nov., v. 51, No. 5, 861–78, 4 figs. [29 refs.]

VÁLEK, A., NEUWIRTOVÁ, R. & CHYTIL, M. **Treatment of Acute Renal Failure in the Course of Weil's Disease by the Artificial Kidney.** *Rev. Czechoslovak. Med.* Prague. 1959, v. 5, No. 1, 32–9, 1 fig. [29 refs.]

Acute renal failure is the commonest cause of death in Weil's disease. Oliguria may proceed to anuria and to death in uraemic coma between the 7th and 17th day [see *Trop. Dis. Bull.*, 1946, v. 43, 338]: in a minority of patients urinary excretion may return spontaneously and in them a profuse polyuria may result in hypokalaemia.

Demonstrable histopathological changes in the kidney are limited to the tubules and interstitial tissues, the glomeruli appearing to escape. The tubular epithelium shows swelling, degeneration and necrosis, with numerous casts in the lumen, but tubular obstruction is not observed. Focal infiltration with lymphocytes is seen in the interstitial tissues, and some fibrosis may result. The signs of "chronic nephritis" do not develop. Leptospires may be observed in large numbers within the tubules, but never in the glomeruli. These changes are not specifically confined to Weil's disease, for they are indistinguishable from those seen to follow mismatched blood transfusions and acute haemolysis of other causes.

Modern clearance investigations in Weil's disease have shown a general impairment of renal function, the concentrating power of the kidney being the slowest to return to normal.

The authors are non-committal as to the mechanism of anuria in this disease, but suggest that it is due to hypotension and to a decreased blood flow through the renal cortex, presumably brought about by some vascular shunt mechanism.

The case of a veterinary surgeon aged 27 is described. The patient injured himself while dissecting an infected guineapig and ignored the wound. 10 days later an acute febrile illness began, which was characterized by muscular pains, vomiting and productive cough. Sulphonamides and penicillin were given. On the 6th day of the illness oliguria and jaundice appeared, and the

urinary flow ceased completely within a few hours. The blood pressure was 100/70 mm. Hg. After 3 days' treatment with antibiotics, vitamins and repeated doses of immune serum, urinary flow returned but the blood urea continued to rise and hypokalaemia developed. The serum bilirubin reached 48 mgm.%. Dialysis by means of an artificial kidney was begun, and led to a prompt fall in the blood non-protein nitrogen and to marked symptomatic improvement. However, haemorrhage from the sites of cannulation occurred and the procedures had to be stopped after 1 day.

Following the resumption of the Bull type of regime the non-protein nitrogen level continued to rise, but urinary flow increased and the clinical condition did not deteriorate, though extra potassium still had to be given. Ultimate recovery was complete, and by the 50th day a maximum urine specific gravity of 1,020 was attained.

[Dialysis may just have swayed the balance in favour of this patient, but the technique is clearly complicated by the marked haemorrhagic tendency and by other effects of hepatic necrosis.] *J. H. Walters*

VIRUS INFECTIONS

The order is:—*General; Psittacosis-Lymphogranuloma group; Pox group; Respiratory group; Enteric group; Arthropod-borne group; Others.*

DAVIS, D. E. & WATKINS, J. R. **The Effect of Chlortetracycline on the Immunological Response of Turkeys infected with Ornithosis.** *J. Infect. Dis.* 1959, Jan.–Feb., v. 104, No. 1, 56–60.

"Ornithosis was transmitted from infected to healthy poults, but 100 g of chlortetracycline per ton of feed prevented spread.

"Chlortetracycline when fed at the rate of 200 g per ton of feed prevented mortality and suppressed the development of lesions in ornithosis-infected poults. Reinoculation of other poults given the same treatment revealed that they were susceptible to infection. Indirect complement-fixation tests did not demonstrate antibodies in the serums of these poults.

"Adult turkeys that recovered from ornithosis, with or without treatment with chlortetracycline, developed significant levels of indirect complement-fixation antibodies and were resistant to subsequent challenge."

KARUNARATNE, W. A. **An Outbreak of Smallpox in Ceylon in 1957.** *Med. Officer.* 1959, Nov. 20, v. 102, No. 21, 263–5, 1 fig. [13 refs.]

This paper is a summarized version of a more detailed account (see *Trans. Soc. Med. Officers of Health, Ceylon*, 1959, v. 18, 23). The island of Ceylon is at special risk to the importation of smallpox by reason of its proximity to the endemic areas of the disease in India The outbreak here described occurred in 1957 and involved 19 recognized cases with a mortality of 38·6%.

It started in the town of Kalpitiya, whence a family of 7 had visited India on 7th January. After their return all were vaccinated on 21st January. One, a boy aged 7, gave a typical primary response. He was said to have been vaccinated when 2 years old, but no scars were present. On 25th January this boy developed fever and a mild rash which were diagnosed as chicken pox. The author does not entirely accept this as the origin of the outbreak, and retrospectively the diagnosis was changed to "generalised vaccinia".

The first recognized case of smallpox, in a woman who lived within 100 yards of this family, had its onset on 10th February, although the diagnosis was not made until 23rd February. This woman first carried the infection into hospital in Colombo. Here 3 patients who died in purpuric states gave rise to at least 4 other cases. One of the fatal cases is claimed "to furnish positive evidence on the lack of protection provided by revaccination". This patient had 3 large scars [age not stated], was "successfully vaccinated" on 25th February, developed a rash on 6th March and died on 10th March. Though doubt is expressed as to the nature of the post-vaccinal reaction in this case, the author expresses surprise at the fatal issue [but too much must not be expected from vaccination performed as late as 5 days after exposure to infection]. As usual, special difficulties in diagnosis arose from the very toxic and the very mild cases.

This outbreak emphasizes the need for the protection, by recent successful vaccination, of dwellers in Ceylon who make visits to India, and of all those whose work, especially in hospitals, may, however rarely, bring them into contact with smallpox. *J. Pickford Marsden*

ELLIOTT, W. D. **Vaccinial Osteomyelitis.** *Lancet.* 1959, Dec. 12, 1053–5, 2 figs.

The occurrence of destructive changes in the metaphyseal ends of long bones, in young children in the late acute or convalescent stages of smallpox has lately been reported, and the literature reviewed by COCKSHOTT and MACGREGOR [this *Bulletin*, 1959, v. 34, 16]. [Most reports concern African or Indian children and no recent reference has come from the United Kingdom, even during the variola minor epidemic years 1919–1932.]

The case described here occurred in a baby 5 months old who developed signs related to the left elbow 2 weeks after vaccination [result not stated] in that arm. X-ray examination at first showed no abnormality but later bone destruction and periosteal new bone formation at both ends of the humerus were apparent. Deformity developed as the osteomyelitis resolved.

The diagnosis of vaccinial osteomyelitis was a presumptive one based on the similarity of the clinical course to that of cases in which the cause was attributed to the virus of smallpox.

J. Pickford Marsden

LIBRACH, I. M. **Congenital Chickenpox.** *Postgraduate Med. J.* 1959, Oct., v. 35, No. 408, 575–7. [30 refs.]

The author discusses the mechanism of transplacental infections and summarizes the rather scanty literature

on neonatal varicella. He records a further possible case in an infant who was born on the 10th day of his mother's chickenpox rash. 4 days later the baby developed a few papules on the legs and trunk. These did not progress. Recovery was uneventful in mother and child. *J. Pickford Marsden*

BAIRD, W. L., Jr. **Chickenpox Pneumonia treated with Steroids.** *U.S. Armed Forces Med. J.* 1959, Nov., v. 10, No. 11, 1359–62, 1 fig. [24 refs.]

The author has traced in the literature 54 cases of primary chickenpox pneumonia: of these 9 proved fatal: in 3 cases steroids were used [this *Bulletin*, 1953, v. 28, 684; 1957, v. 32, 228; 1959, v. 34, 314]. The case here described occurred in a white soldier aged 28 years. Mild respiratory symptoms developed 4 days before malaise and the appearance of a chickenpox rash. Two days later the man was acutely ill; temperature 103°F. skin eruption extensive; no abnormal signs in lungs. White count was never above 10,000 per cmm. Cold agglutination tests and serological tests for Q fever, syphilis and psittacosis were negative. Chest X-ray, however, showed multiple, bilateral, nodular pulmonary infiltrations, which took about 1 month to clear completely. The patient's condition rapidly deteriorated: his temperature rose still more, the blood pressure fell and he became dyspnoeic, cyanotic, and unresponsive. In addition to sponging, aspirin, intravenous fluids, oxygen, penicillin and chloramphenicol he was given intravenous hydrocortisone, 300 mgm. in 24 hours, followed by prednisone for 4 days—80, 80, 40 and 40 mgm. respectively.

On this régime the patient made a rapid recovery. It is not possible to say dogmatically which, if any, part of the treatment proved decisive. The diagnosis of chickenpox pneumonia is usually a presumptive one and the illness usually ends abruptly. In this case it can at least be said that steroid therapy appeared to be without harmful effort. *J. Pickford Marsden*

AGARWAL, B. L. **Encephalomyelitis following Chickenpox.** *J. Indian Med. Ass.* 1959, Nov. 1, v. 33, No. 9, 381.

Report of a case.

ELLISON, S. A., CARTON, C. A. & ROSE, H. M. **Studies of Recurrent Herpes Simplex Infections following Section of the Trigeminal Nerve.** *J. Infect. Dis.* 1959, Sept.–Oct., v. 105, No. 2, 161–7, 4 figs. [16 refs.]

In people subject to the recurrent lesions of herpes simplex, local vesicular eruptions are known to appear a few days after pre-ganglionic section of the trigeminal nerve. An attempt has been made to relate serum antibody level to the recurrence of herpetic lesions in a group of people undergoing trigeminal section to relieve neuralgia.

All the patients possessed either neutralizing or complement-fixing antibodies, or both, before operation. After operation, 18 of 26 patients developed herpes in the denervated area; virus was isolated from the peripheral lesions of 8 out of 12. Antibody levels did not differ significantly in those who did and those who did not develop herpes. The rise in titre observed after open infection was irregular, and the severity of the lesions was not related to initial antibody levels. Although virus, recognized by the pocks it produced on the chorioallantoic membrane of the chick embryo, was isolated from their lesions, it was not demonstrated in samples of Gasserian ganglia from 2 people. This is cited, incidentally, as evidence that the neuralgia is not caused by the virus itself, as has sometimes been suggested. *Janice Taverne*

FLECK, D. G. **Respiratory Virus Antibodies in Pregnant Women in South-West Wales.** *Monthly Bull. Ministry of Health & Pub. Health Lab. Service (directed by Med. Res. Council).* 1959, Sept., v. 18, 155–8, 2 figs.

"Antenatal sera from urban and rural areas were examined for complement-fixing antibodies to influenza A, B and C viruses, the Sendai-haemadsorption group of viruses, the psittacosis-lymphogranuloma-venereum group of viruses, *Rickettsia burneti*, adenovirus, and mumps virus. No significant difference was noted between the two groups."

SAMPAIO, A. & CAEIRO, F. de M. Contribuição para o estudo da epidemiologia da gripe através de um inquérito por "amostra representativa da população" da cidade de Lisboa. [**Study of the Epidemiology of Influenza by Means of Survey of a Sample of the Population of Lisbon**] *Bol. Serviços de Saúde Pública.* Lisbon. 1958, Oct.–Dec., v. 5, No. 4, 383–491, 3 graphs & 1 map.

The English summary appended to the paper is as follows:—

"The epidemiology of influenza was investigated in 3,076 families, a sample of the population of the city of Lisbon. The sample was chosen in collaboration with the National Institute of Statistics and includes people from all social-economic levels so that these factors could be taken into account in the evaluation of the incidence of the influenza epidemic of 1957.

"In the investigation made, nurses, previously instructed on 'flu' epidemiology, visited these families and the data thus collected were grouped by the mecano-graphic services of the Institute.

"It was found that in August 1957 there appeared cases of influenza that increased from September onwards reaching their peak in the second week of October. This coincided with the closing down of schools on October 8th, the High Schools having opened on October 1st, and Primary Schools on the 7th. The epidemic lasted about two months with high prevalence of the disease during two weeks.

"Of the 11,777 people that made up the 3,076 families under study, 4,860 fell ill, a high rate of 41.2%.

"All the age groups were affected but the greatest incidence was observed in young people from 5–19 years and predominantly in the 10–14 age group.

These data seem to show that differences examined in the various age groups are not due to their previous immunity but to their social-economic conditions.

"Those under study were divided, according to their social-economic status, into five groups: very bad, bad, reasonable, good and very good. It was shown that the incidence of the disease decreased as the standard of living increased: thus 60.1% for the former and 31.7% for the latter.

"Evaluating these differences, grouping the data of the classes very bad and bad, good and very good it was observed they have a statistical significance ($p < 0.001$).

"The medium duration of the disease was 6.4 days. It was longer in the lower social levels (6.7 days) than in the higher (5.9 days); in women (6.6 days) longer than in men (6.1 days), and in those over fifty longer too (7.8 days).

"The differences observed are statistically significant ($p < 0.001$).

"The percentage of complications observed was 27.9, the most frequent being asthenia (15.2%) and bronchitis (11.2%) both longer than usual.

"People in the age group 20–40 were the most affected with bronchopulmonary complications.

"47.3% of the people who fell ill had no medical assistance.

"In the group with medical attention 56.8% had a private doctor, 39.2% were treated in several public services and 3.9% in Hospitals."

SAMPAIO, A., AYRES, Laura & NUNES, Maria I. P. Aspectos laboratoriais da epidemia de "gripe asiática". [**Laboratory Aspects of the Asian Influenza Epidemic**] *Bol. Serviços de Saúde Pública. Lisbon.* 1958, Oct.–Dec., v. 5, No. 4, 493–518, 1 graph.

The English summary appended to the paper is as follows:—

"The first cases of Asian influenza in Portugal were diagnosed in August 1957, the infection having been introduced by passengers from a ship back from African ports on board of which several cases had occurred.

"From August till late September several cases of influenza were observed all over the country, with small epidemic outbreaks in some holiday camps.

"At the end of September the epidemic began abruptly reaching its highest point, in Lisbon, on the second week of October.

"Seventeen strains of influenza virus were isolated from August 1957 to March 1958 all of which belonged to the Asian type.

"For diagnostic purposes hemagglutination inhibition and complement fixation tests were made.

"The first gave different results according to the strains used as antigens. The A/Singapore/1/57 strain proved to be a bad antigen since the majority of the reactions were negative, in both acute and convalescent sera.

"On the contrary, the A/Lisbon/2/57 strain proved a good means of diagnosis, easily showing up the antibodies.

"The complement fixation test using soluble antigen was shown to be a good process of diagnosing the disease.

"Serological studies were made before the beginning of the epidemic in our country, with sera of persons over seventy years old. No antibodies inhibiting hemagglutination were found in these for the A/Singapore/1/57 and A/Japan 305/57 E_5 F_6 M_6 E_6 strains used as antigens.

"The percentage of inapparent infections was ascertained by using the C. F. T., considering as a sign of recent infection a titre equal or above 40. Percentages of 38.8 for children and 33 for adults were obtained.

"Working together with the Central Laboratory of Veterinary Pathology 50,000 doses of vaccine were prepared.

"Its antigenic value was observed in volunteers and mice, being weak in both of the groups under study. It was not possible to assess its prophylactic value as the first doses were obtained when the epidemic was already taking its course."

COFFEY, Victoria P. & JESSOP, W. J. E. **Maternal Influenza and Congenital Deformities. A Prospective Study.** *Lancet.* 1959, Nov. 28, 935–8. [14 refs.]

This investigation was carried out in 3 Dublin maternity hospitals—Rotunda, National Maternity and Coombe hospitals—and covered one year from October 1957 which included the outbreak of Asian influenza. All women attending the antenatal clinics were asked if they had had an attack of influenza during their pregnancy. For each woman who had had influenza another woman attending the clinic on the same day and at about the same stage of pregnancy who had not been attacked was taken as a control. Each group finally consisted of 663 women. Among the mothers who had had influenza 3·6% of the births were malformed compared with 1·5 among the controls. The distributions of abnormalities between the 3 hospitals were different; in the Rotunda 2·7% of the births of both groups of patients were deformed, in the National Maternity hospital 3·9% of the mothers who had had influenza and 1·0% of the controls had babies with deformities and in the Coombe hospital the proportion of defective babies was 4·4% for the mothers who had had influenza and 0·7% for the controls. The abnormalities were almost entirely of the central nervous system and anencephaly was the commonest. The distribution of abnormalities by the period of pregnancy when influenza was contracted was 7·4% for the first, 4·3% for the second and 2·0% for the third trimester. The infected mothers had 18 stillbirths (6 malformed), and the controls had 19 (3 malformed). The number of babies with a birth weight under 2,500 gm. was 60 (14 malformed) for the group of mothers who had influenza and 52 (4 malformed) for the control group. The authors briefly discuss the mechanics by which the influenza virus can damage the embryo.

[For a previous study by the authors, see this *Bulletin*, 1955, v. 30, 390.] *W. J. Martin*

See also p. 228, VIGLIANI & FINULLI, [**Increased Resistance of Silicotics to A/Asian Virus Infection.**]

NÁSZ, I. & TÓTH, M. **Frequency Distribution of the Complement Fixing Antibodies to Adenoviruses in Several Groups of the Hungarian Population.** *Acta Microbiologica.* Budapest. 1959, v. 6, No. 3, 203–7, 2 figs. [18 refs.]

"Serum specimens from 426 Hungarian subjects belonging to four groups have been tested for complement fixing antibodies to adenoviruses. Group I consisted of children under orthopaedic treatment following an attack of poliomyelitis; group II included patients with pulmonary tuberculosis, between 10 and 20 years of age; groups III and IV consisted of healthy persons aged 18–20 years and more than 20 years, respectively. The proportion of sera with positive reaction was 48.2 per cent in group I, 24.5 per cent in group II, 14.7 per cent in group III and 13.6 per cent in group IV. There was some inverse relation observable between age and this proportion. The highest titre was 1:32 in each of the groups I, II and IV, and 1:8 in group III."

LARSEN, K. A. **Diffuse Progressive Interstitial Fibrosis of the Lungs.** *Acta Path. et Microb. Scandinavica.* 1959, v. 45, No. 2, 167–85, 4 figs. [Numerous refs.]

Following the description of diffusive progressive fibrosis of the lungs by HAMMAN and RICH in 1934, about 30 other cases have been reported by various authors. Most cases were acute but a few more chronic cases have been reported. Other authors have reported cases which bear a resemblance, but are probably not identical with the condition.

5 cases are reported in this paper, all with post-mortem findings. The ages were 63–78 years. All had lung symptoms for a number of years, but the progressive dyspnoea lasted 5 weeks in 1 patient, and 1–4 years in the others. The X-ray findings were mainly small scattered opacities. Clinically in addition to the progressive dyspnoea, febrile episodes occurred, and the terminal event was progressive respiratory failure. In 3 patients the introductory symptom was a febrile illness. Pathologically, the main features were marked increase in the connective tissue elements with small cystic spaces. In the affected areas the respiratory epithelium had practically disappeared.

An interesting discussion on the pathogenesis follows. The author argues in favour of respiratory virus infection as the primary process affecting the bronchi, followed by extensive epithelial necrosis, and often of necrosis of the interstitial mesenchymal tissue. He is not impressed by the allergic theory. He compares the process with the cirrhosis of the liver which sometimes follows virus hepatitis.

A feature is the full bibliography of the literature which will be of great value to anyone studying the subject. The article is interesting and well presented.

F. H. Young

NOORDAM, A. L., SCHRICKX, A. M. & DEKKING, F. Antistoffen tegen poliomyelitis bij Amsterdamse kinderen. [**Antibody against Poliomyelitis in Amsterdam Children**] *Nederl. Tijdschr. v. Geneesk.* 1959, Oct. 24, v. 103, No. 43, 2150–55, 10 figs. English summary.

A study was made of the incidence of naturally acquired antibody in 1,654 children under the age of 12 in Amsterdam, before vaccination against poliomyelitis. The children were divided into 4 groups according to the part of the town they came from, *i.e.* according to social class. Neutralizing antibodies against the 3 types of virus were detected by the colour test in plastic trays, with a line of epithelial cells derived from a human foetus.

It was found, as was expected, that the percentage of children with antibodies was higher at all ages in families from the lower social grades. However, the differences within the 2 better-off groups and the 2 poorer groups were small, suggesting that factors such as the size of family and of their house, and their way of living were more important in determining the spread of virus than prosperity alone. The incidence of antibodies was the same in both sexes. Most of the children first went to school at 4 years, but there was no sudden increase in the incidence of antibodies after that age.

The detailed results of this methodical survey are given. The morbidity rates for poliomyelitis closely parallelled the state of immunity of the population at all ages (except under 2 years, perhaps owing to a smaller chance of infection or a higher natural resistance). The authors compare their results with those of a survey of a similar population in Lower Saxony (WOHLRAB *et al.*, *Deut. med. Woch.*, 1957, v. 82, 1922) in which the same pattern of natural immunity and disease, related to age, was found. These results contrast with work done in Stockholm [this *Bulletin*, 1957, v. 32, 557]. Although the incidence of antibodies was no lower than in the Dutch and German children at the different ages, the total morbidity was higher, and 50% occurred over the age of 19 in Sweden, but under the age of 9 in Holland and Germany.

Janice Taverne

SACHTLEBEN, P. & SCHELLENBERGER, A. Zur Frage der Poliomyelitisinfektion von Klinikpersonal. [**The Question of Poliomyelitis Infections among Hospital Personnel**] *Deut. med. Woch.* 1959, June 19, v. 84, No. 25, 1142–5, 1160, 2 figs. [29 refs.]

An investigation was made of the titres of complement-fixing antibody during the second half of 1956 in 65 members of the medical and nursing staffs of a children's hospital in Munich, and also in 2 control groups consisting of 44 children admitted to hospital during the early part of the same period with various diagnoses and 71 sera from adults sent for Wassermann tests. The complement-fixation tests were carried out by HENNESSEN's method [this *Bulletin*, 1956, v. 31, 110]. In the test group complement-fixing antibody was found in 34% of 132 sera, 35% of which contained

type I, 30% type II and 35% type III antibody, and in 47% of the control group of children and 18% of the adults. *D. J. Bauer*

Majori, L., Farisano, G., Gamba, F. & Gasparini, V. L'epidemia di poliomielite di terrangera (Padova) nell'autunno 1958. [**The Polio Epidemic of Terranegra (Padua) in Autumn 1958**] *Igiene Moderna.* 1959, May–June, v. 52, Nos. 5/6, 331–41, 1 map & 1 graph. English summary (8 lines).

Terranegra is a small rural neighbourhood of 2,500 inhabitants in the Commune of Padua. This outbreak of paralytic poliomyelitis occurred in October and November and involved 13 children aged 6 months to 6 years. There had been no cases in 1957, one case in October 1956, one in May 1955 and none in 1954 or 1953. In 1958, other parts of Italy have shown a rise, and a change of season, in the incidence of the disease.

The investigation which is here reported was extended to family contacts. 3 specimens each of faeces for virus and blood serum for antibodies were taken wherever possible, namely, as soon as the disease was recognized and then about 30 and 50 days later. The results of laboratory examination are summarized in a comprehensive table and the topographical distribution of the cases is shown by a plan.

Stool specimens were taken from 11 out of 13 patients and Type 1 virus was isolated from 8 (72·72%). 3 went on excreting the virus for over 30 days but were negative by the 50th day; the other 5 had stopped excreting by the 30th day. All 8 showed antibodies to Type 1 in a progressively rising titre.

35 family contacts were examined and Type 1 virus was isolated from 14 (40%). 4 were still excreting virus by the 30th day and one of these by the 50th, but in 2 other positive contacts only one first specimen could be examined. As with the patients, there was a progressively rising titre of specific antibodies in the blood serum.

Some antibodies against Type 2 virus were found in both patients and contacts.

2 patients died, giving a case fatality of 15·38%.

In all cases but one there was evidence of contact with a certain children's home, sometimes only indirectly through others who attended the home.

At the relevant time, 5 of the contacts showed evidence suggestive of non-paralytic infection, with fever, constipation, sore throat and, in one case, epistaxis.

Environmental sanitation was of a low standard, many obtained their water supply from shallow wells and nightsoil was disposed of by conservancy means.

J. Cauchi

Galpine, J. F. & Wilson, W. C. MacC. **Occurrence of Myocarditis in Paralytic Poliomyelitis.** *Brit. Med. J.* 1959, Dec. 19, 1379–81, 2 figs. [18 refs.]

This article describes 5 fatal cases of poliomyelitis: in all, myocarditis was shown on histological examination. There was hyperaemia, structural distortion, fragmentation of muscle and cellular infiltration, both perivascular and diffuse: lymphocytes and large monocytes are commonly described but in this series polymorphonuclear leucotypes were also prominent. The literature of the subject is briefly reviewed.

A. B. Christie

Škovránek, V. & Roudný, J. **Vaccination against Poliomyelitis in Czechoslovakia.** *Rev. Czechoslovak Med.* Prague. 1959, v. 5, No. 2, 97–108, 5 figs. [16 refs.]

This paper is an interesting review of poliovaccination in Czechoslovakia. In 1957 a large-scale vaccination campaign was initiated with Canadian Salk type vaccine administered intradermally. 87% of the children aged 1–7 received 2 doses of vaccine and nearly 40% of those 8–14. The vaccine was considered safe and about 70% effective. [This was not based on a controlled trial, but on analysis of the attack rate in vaccinated and unvaccinated children up to 7 years.] In 1958 a booster dose was given, and the vaccine programme made more widespread up to 14 years. Also new primary immunizations with 2 × 1 ml. doses subcutaneously was started in 7-month-old infants. In 1958 the morbidity from poliomyelitis reached the lowest level for 20 years. Although these results were encouraging [despite the intradermal route of inoculation, found elsewhere to result in poor antibody responses] it was thought that, in view of the experiences in Israel where a major epidemic occurred in a vaccinated population, and of fears about the duration of immunity, the results were still unsatisfactory. A trial of Sabin's attenuated vaccine was therefore undertaken, all 3 types being successively administered to 143,000 children previously vaccinated 3 times with Salk vaccine intradermally. It will therefore be possible to compare the response of children (a) vaccinated with 3 doses of Salk vaccine intradermally, (b) vaccinated as above but with a fourth booster dose of Salk vaccine, subcutaneously, (c) vaccinated with 3 doses of Salk vaccine followed by Sabin's strains of attenuated poliovirus. *A. J. Beale*

Rosa, A. & La Placa, M. Osservazioni sull'effetto immunizzante di piccole dosi di vaccino antipoliomielitico inoculate per via intradermica in soggetti adulti. [**The Immunizing Effect of Small Doses of Poliovaccine given Intradermally to Adults**] *Riv. Italiana d'Igiene.* 1959, May–June, v. 19, Nos. 5/6, 143–8, 1 fig. English summary (6 lines).

The authors consider that in the quite near future it is not improbable that because of diminishing natural immunity it may be necessary to immunize adults, so they tried the effect of small doses of vaccine on 10 persons aged between 20 and 53 years. The vaccine used was a commercial Salk-type containing Brunhilde, MEF-1, and Saukett strains. All the patients were given 3 injections of 0·2 ml. intradermally, the second 30 days after the first, and the third 45 days after the second. Blood samples were taken before the first injection, at the time of the second and third injections, and 45 days after the third injection and the results are set out in a table. The sera were tested in dilutions from 1:8 to 1:2048 with 100 CPD50 of virus for neutralization as the criterion.

The results were: (a) there was only a slight local reaction which became normal in 3–4 days; (b) persons with antibodies before the first injection responded well to all 3 types; (c) in 3 persons without antibodies for types 1 and 2 the first injection caused these to appear in low titre, but for one person without antibodies to type 3 only after third injection. In 3 persons for type 1, 2 for type 2, and 5 for type 3 the titre after the third injection was less than after the first. The authors could give no reason for this, but conclude that in adults natural immunity can be notably reinforced by the intradermal injection of 0·2 ml. poliovaccine. *W. K. Dunscombe*

HORSTMANN, Dorothy M., NIEDERMAN, J. C., RIORDAN, J. T. & PAUL, J. R. **The Trial Use of Sabin's Attenuated Type 1 Poliovirus Vaccine in a Village in Southern Arizona.** *Amer. J. Hyg.* 1959, Sept., v. 70, No. 2, 169–84, 6 figs. [21 refs.]

The authors have already described a series of valuable, carefully controlled, small scale trials of attenuated poliovirus (LSc type 1 strain developed by SABIN) [this *Bulletin*, 1959, v. 34, 1085]. Because attenuated poliovaccine will probably be cheaper than killed vaccine it has been thought that it may find its first application in underdeveloped areas. The present study was carried out in a community (Guadalupe, Arizona) of low socio-economic status. 6 children under 5 were fed 10^5 TCD50 of the LSc strain of type 1 poliovirus. Prior to feeding only 2 of the children lacked type 1 antibodies and 1 of these became infected, excreting virus for 30 days and showing a good antibody response. 2 of the remaining 4 children became infected, excreting virus and showing an antibody response. These 2 infected children had prefeeding antibody titres to type 1 of 1/64 and 1/128 while the 2 not infected had titres of 1/32 and 1/8. Limited tests on the excreted viruses showed they were not virulent for monkeys inoculated intracerebrally with 10^6 TCD50. Tests on family and community contacts showed no spread of virus but only 1 family contact of an infected child was susceptible.

The disappointing results of this trial are ascribed to the high level of immunity to all 3 types of poliovirus in the community. Thus the pattern of antibody prevalence was similar to that found in Egypt or Morocco. All persons over 5 had antibodies to all 3 types and only 10 out of 32 under 5 lacked antibodies to type 1. The dose of virus used was probably too low, thus the authors previously found that $10^{7·4}$ TCD50 is a more generally effective dose. Another contributing factor may have been the prevalence of other enteroviruses. The trial was carried out in February yet 77% of the children under 5 were excreting an enterovirus at least once during the 6 weeks of observation. Samples of faeces from privies in general reflected the viral flora of the families using the privy. Many viruses, including 26 type 1 and 36 type 3 polioviruses, were isolated from flies. The type 3 strains certainly and the type 1 probably were wild strains, although 3 of the 7 type 1 strains did not cause paralysis in monkeys by the intracerebral route.

[This trial indicates some of the practical problems involved in the use of attenuated poliovaccine in an underdeveloped area.] *A. J. Beale*

STUDER, A. Die Hepatitis epidemica. Eine Epidemie in einer abgeschlossenen Talschaft. **[An Epidemic of Infectious Hepatitis in a Secluded Valley]** *Ztschr. f. Präventivmed.* Zurich. 1959, Oct., v. 4, No. 10, 336–61, 10 figs.

In this paper a considerable account of the history of infectious hepatitis and its aetiology, epidemiological characters and clinical picture precedes a description of some of the epidemic experience of the disease in Switzerland recorded in the literature since the middle of the 19th century. There follows a detailed report of an epidemic which occurred in 1953–55 in a Swiss valley district where among a population of 2,680 persons 304 cases of hepatitis were discovered. Investigations were made of topographical circumstances, housing conditions, water supply, drainage, food supply and the possibilities of spread of infection in families, at school and at work. The findings are fully described with illustrative figures.

The first cases of hepatitis were noted towards the end of 1953 but the greatest prevalence of the infection was experienced in the middle months of 1954 after which only occasional cases were reported. Most of the cases were in schoolchildren 6 to 16 years of age and both sexes were equally affected. No origin of the infection appears to have been discovered. From the evidence it appears that the spread of the disease was due to close contact in the home and at school. The incubation period of the disease was 3–5 weeks.

There is some discussion of preventive measures.

M. E. Delafield

ANDERS, W. & KIMA, T. Zur Epidemiologie der Hepatitis epidemica in Deutschland. **[On the Epidemiology of Epidemic Hepatitis in Germany]** *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 176, Nos. 1/2, 1–34, 19 figs. [Numerous refs.]

This paper on the epidemiology of epidemic hepatitis in Germany contains a long and detailed account, with many tables and some maps, of the incidence and character of the disease particularly in the last 7 years. The incidence has been high in this period—over 37,000 cases in 1957 in the East German Republic are given in a table, the next highest figures having been about 25,000 in Czechoslovakia and about 15,000 in the United States. The epidemiological features of the disease and its geographical distribution are fully described with an account of the investigation of some epidemics. Explosive epidemics were considered usually to be due to contaminated drinking water. Contact epidemics also occurred. Seasonal incidence was greatest in late autumn and winter. The highest incidence was in the age-group 11 to 15 years; males and females were equally affected and mortality was highest in the age group 50–80 years. The immediate case mortality was very low but considerable stress is laid on the late complications and sequelae of the disease which according to some published work are

numerous and require a systematic follow-up of all patients to ascertain their frequency.

Some account is given of the methods that are most important for the prevention and control of the disease with mention of the value of administration of gamma globulin with which Czechoslovakian workers have reported success in individual protection.

M. E. Delafield

DENNIS, J. M. 1955–56. **Infectious Hepatitis Epidemic in Delhi, India.** *J. Amer. Water Works Ass.* 1959, Oct., v. 51, No. 10, 1288–96, 1 fig. Discussion 1296–8 [WOLMAN, A.].

This paper presented at the annual conference of the American Water Works Association held at San Francisco, California, in 1959 is based on a series of articles published as a special issue of the *Indian Journal of Medical Research* in 1957 [this *Bulletin*, 1957, v. 32, 926]. It concerns the explosive epidemic of infectious hepatitis which started in Delhi, India, and is the largest "that has ever occurred". It began in the first week of December 1955 and lasted about 6 weeks. Some 7,000 cases of the disease were reported to the health authorities and a sample survey indicated a total number of 29,300 cases of jaundice in the population of 1,700,000. The total number of infections was estimated at 1,000,000. "This was the first well documented epidemic of infectious hepatitis involving a treated water supply" and "should thus be of unusual interest to sanitary engineers and water utility operators".

The salient features of the water sources, water treatment, water pollution during November 1955, chemical analyses and chlorine residual determination are described. Unfortunately no record of quantitative bacteriological data has been made available.

This epidemic of infectious hepatitis was caused by a combination of unfortunate circumstances in that "receding floods in the Jumna River permitted heavy contamination of the raw water at the Chandrawal water treatment plants. At its worst the raw water may have contained as much as 50 per cent sewage". These treatment plants "were able to produce well clarified water which was evidently bacteriologically safe, as there was no reported increase in typhoid and dysentery above the high endemic levels following the heavy pollution. Disinfection with low combined chlorine residuals was, however, evidently inadequate to protect against the infectious-hepatitis virus".

The paper is followed by a valuable discussion.

M. E. Delafield

See also p. 220, CLARKE & LANG, **Enteric Viruses in Water.**

HEISTÖ, Helge & JULSRUD, A. C. **Silent Carriers of Hepatitis Virus B.** *Acta Med. Scandinavica.* 1959, July 25, v. 164, No. 4, 349–55, 2 figs.

The serious problem of the transmission of hepatitis by blood donors is discussed, and 2 instances are given of such transmission to 6 and 4 persons respectively by "silent" carriers of the virus, that is, by blood donors without a history of liver disease and with completely normal liver function tests [this *Bulletin*, 1954, v. 29, 1137: 1956, v. 31, 889]. Both of these donors had apparently been silent carriers in this sense over a period of almost 4 years. The difficult question is posed: what should be done to detect such a donor when a patient develops hepatitis some 2 months or so after blood transfusion? The answer given is as follows.

All the possible donors involved should be examined by means of a series of liver function tests, and careful enquiry should be made into the histories of all previous donations made by these donors. If abnormal liver tests, or another case of hepatitis apparently transmitted by the same donor are found, that particular donor should be excluded from subsequent donations. If there is only one donor and no other source of infection is found, he or she should be excluded even if both modes of examination yield negative results. If there are several possible donors involved, and all the examinations are negative, either all should be excluded from further donation or, if that is not possible, the history of their subsequent donations should be carefully followed in order that the carrier may be detected at the earliest possible moment.

H. Stanley Banks

DE RITIS, F., MALLUCCI, L., COLTORTI, M., GIUSTI, G. & CALDERA, M. **Anicteric Virus Hepatitis in a Closed Environment as shown by Serum Transaminase Activity.** *Bull. World Health Organization.* Geneva. 1959, v. 20, No. 4, 589–602, 8 figs. [28 refs.]

The transaminases are enzymes which transfer amino-groups from amino-acids to keto-acids, thereby converting the latter into amino-acids. Of particular interest for the diagnosis of liver disease are the glutamic-oxalacetic transaminase (GOT) which produces aspartic acid and the glutamic-pyruvic transaminase (GPT) which produces alanine. Normally these 2 enzymes are present at low concentration in the serum, the latter (GPT) at a slightly lower concentration than the first (GOT). In liver disease necrosis of liver cells releases the 2 enzymes into the peripheral circulation and particularly the GPT can rise to concentrations which are several hundred times greater than normal. By using the measurement of the transaminases as a guide it has been possible to show that jaundice is only a crude indication of viral hepatitis and that necrosis can proceed without icterus. The authors have carried out systematic determinations of the 2 transaminases over a period of 60 days in 104 children and 11 adults living together in an institution for children in Rome, where several cases of icteric hepatitis had been observed over the last 2 years. On the assumption that hepatitis was endemic in this environment some 1,000 enzyme determinations were made. 1 of the 11 adults and 31 of the 104 children showed biochemical evidence of hepatitis. Only 1 of the children had frank icteric hepatitis, the others were never jaundiced but a large number showed clinical symptoms such as anorexia, dyspepsia, hepatomegaly and either simultaneously or later positive serum lability tests.

14 of these children showed only a slight increase in enzyme activity, with or without inversion of the transaminase ratio. In 4 the enzyme activity was greatly increased and the transaminase ratio was inversed. One of the two changes or both reverted to normal in the course of the observation period. 1 of these 4 children developed the icteric type of the disease later on and *after* the diagnosis had been made on biochemical grounds. This is the child mentioned above. 13 children were normal initially but developed an increase in transaminase values and inversion of the enzyme ratio in the course of the observation period.

From their finding of a ratio of 30:1 of anicteric over icteric hepatitis the authors conclude that the frequency of hepatitis may be grossly underestimated in institutions for children where the infection is known to be endemic. A plea is made for a more widespread use of transaminase estimation in the diagnosis of the disease.

[For previous studies by the authors, see this *Bulletin*, 1957, v. 32, 1028.] *H. Lehmann*

Bodansky, O., Krugman, S., Ward, R., Schwartz, M. K., Giles, Joan P. & Jacobs, A. M. **Infectious Hepatitis. Correlation of Clinical and Laboratory Findings, including Serum Enzyme Changes.** *J. Dis. Children.* Chicago. 1959 Aug., v. 98, No. 2, 166–86, 5 figs. [36 refs.]

In the course of an attempt to induce passive-active immunity against virus hepatitis in a series of children by the administration of gamma globulin and hepatitis virus under carefully controlled circumstances, the authors had the opportunity to investigate the correlation of clinical manifestations of the artificially induced disease with changes in conventional biochemical tests of liver function and in certain serum enzyme activities. Of particular relevance to their study was the establishment of a set of criteria which would permit a definite diagnosis, not only of cases of hepatitis, but also of subclinical cases. They found that of a set of criteria consisting of fever, jaundice, hepatomegaly, elevated serum bilirubin, elevated thymol turbidity, and elevated serum phosphohexose isomerase or glutamic oxaloacetic transaminase, a cluster of 3 or more only occurred among children who had received virus. The most frequently occurring abnormalities in those with 3 or more findings were jaundice, hepatomegaly, elevated thymol turbidity, and increase of one or other of the enzymes mentioned. The abnormality occurring least frequently was fever, 57%, and elevated isomerase or transaminase occurred most frequently, 95%. The concurrence of 3 or more of the following—jaundice, hepatomegaly, elevated thymol turbidity and elevated serum isomerase or transaminase was therefore considered diagnostic of infectious hepatitis.

The suggestion has been made by Stokes and his co-workers [see this *Bulletin*, 1952, v. 27, 401] that when passive immunization is employed in institutional outbreaks of hepatitis patients continue to be exposed to hepatitis virus. Subclinical infection follows and in turn active immunity develops. In the course of their investigation the authors could elicit no clear evidence that subclinical hepatitis occurred within a period of 30 to 50 days after the conjoint administration of gamma globulin and hepatitis virus. *A. Joe*

Naglo, O., Rydenstam, B. & Silverstolpe, L. **ACTH-Treatment of Acute Viral Hepatitis.** *Acta Med. Scandinavica.* 1959, Oct. 28, v. 165, No. 3, 167–71, 2 figs. [11 refs.]

Adrenocorticotrophic hormone (corticotrophin) was given in a dose of 100 international units daily by intramuscular injection to 25 patients suffering from acute viral hepatitis: another 25 patients were given a placebo. The mean value of serum bilirubin fell more quickly in the corticotrophin-treated group and a bilirubin level of 2 mgm.% and a bromsulphthalein level of 10% were reached more quickly; but the difference was not significant. The thymol value decreased more rapidly in the treated group and the difference was significant at a level of 5%. The possible advantages of treatment with adrenocorticotrophic hormone was counterbalanced by side-reactions. *A. B. Christie*

Scheid, W., Ackermann, R. & Jochheim, K. A. Die Bedeutung der komplementbindenden und der neutralisierenden Antikörper für die Diagnose der Infektionen mit dem Virus der lymphozytären Choriomeningitis. [**The Significance of Complement-Fixing and Neutralizing Antibodies for the Diagnosis of Infections with Lymphocytic Choriomeningitis Virus (LCM)**] *Deut. med. Wochs.* 1959, July 17, v. 84, No. 29, 1293–6, 1323–4. [13 refs.]

The titres of neutralizing and complement-fixing antibodies have been determined in sera taken at intervals up to 5 years from 15 patients suffering from infection with lymphocytic choriomeningitis virus. Complement-fixing antibody was present in the first or second week of illness in 4 instances, attained a maximum titre after 3–4 weeks, and usually disappeared after 3–6 months although it was still present after 4 years in 1 instance. Neutralizing antibody appeared in some cases in the first 2 weeks of illness, but usually after 5–6 weeks reaching a maximum index of nearly 5 log units after 4–6 months and persisting for 5 years or more. There was no correlation between the behaviour of either type of antibody and the severity of the disease.

D. J. Bauer

Fields, William S. & Blattner, Russell J. [Compiled & Edited by.] **Viral Encephalitis. A Symposium. Fifth Annual Scientific Meeting of the Houston Neurological Society, Texas Medical Center, Houston, Texas.** pp. ix + 225, 29 figs. 1958. Springfield: Charles C. Thomas, Bannerstone House, 301–327, East Lawrence Avenue, Ill. U.S.A. [52s. 6d.]

This symposium gathers together papers on the aetiology, epidemiology, laboratory and clinical diagnosis, sequelae, pathology and vaccine prophylaxis of viral encephalitis—mainly as it occurs in the United States.

J. CASALS (pp. 5–21) describes the serological classification of the arborviruses and discusses in detail the occurrence of inapparent infections with encephalitis viruses. Inapparent infections with eastern equine virus appear to be uncommon and with western equine virus only a little more so. On the other hand, Japanese encephalitis has been estimated to cause 500–1,000 inapparent infections for each case of encephalitis and they may also be common with St. Louis virus.

E. H. LENNETTE (pp. 22–45) discusses the epidemiology of western equine and St. Louis encephalitis in California where they are important diseases and have been intensively studied. They occur in areas with high summer temperatures and extensive irrigation. Both diseases occur in the summer but the peak incidence of western equine is in July, that of St. Louis in September. Western equine encephalitis is commoner in young children than St. Louis, while both are commoner in males than females.

The clinical and laboratory diagnosis of virus infections of the central nervous system are discussed in detail by F. C. ROBBINS (pp. 46–68), the salient points being tabulated. For diagnosis of enteric virus infections, isolation of the agent from faeces is the method of choice, although such isolations may not be the cause of the disease during which they are made. The difficulties of the differential diagnosis of encephalitis are detailed.

The sequelae of viral encephalitides are discussed by K. H. FINLEY (pp. 69–94). Motor, intellectual and emotional disorders occur. In eastern and western equine encephalitis the incidence is highest in infants. Sequelae are infrequent in St. Louis encephalitis. A long-term follow-up study in California is described.

W. HAYMAKER, Margaret G. SMITH, L. VAN BOGAERT and C. DE CHENAR (pp. 95–204) in a long, detailed, illustrated paper, discuss the pathology of virus diseases characterized by nuclear inclusions. Herpes simplex and subacute inclusion encephalitides are described in detail and the literature comprehensively reviewed.

In the last paper, H. R. COX (pp. 205–225) reviews the treatment and prophylaxis of rabies by antiserum and vaccine, especially chick and duck embryo vaccines. The effects of Salk vaccine on the control of poliomyelitis in the United States are reviewed. The avirulent vaccine strains of poliovirus are described and some of the early vaccination trials with live vaccine are discussed.

This book is a good review of virus encephalitis for non-virologists although, as the symposium was held in March 1957, it is not now entirely up-to-date.

C. E. Gordon Smith

BRODY, J. A., BURNS, K. F., BROWNING, G. & SCHATTNER, J. D. **Apparent and Inapparent Attack Rates for St. Louis Encephalitis in a Selected Population.** *New England J. of Med.* 1959, Sept. 24, v. 261, No. 13, 644–6, 1 fig. [12 refs.]

This is a study of the complement (3,900 men) of an air base involved in the Cameron County, Texas, outbreak of St. Louis encephalitis in 1957. The whole outbreak involved 120 clinical cases with an attack rate of 163 per 100,000. Between mid-July and late August 11 clinical cases occurred in the Base (an attack rate of 280 per 100,000) and 4 other cases occurred among dependants. There were no deaths or paralytic cases and only slight, but constant, cerebrospinal fluid changes were detected. Comparing acute and convalescent phase sera by complement-fixation a 4-fold or greater rise in titre was demonstrated in 11 of the 15 and suggestive evidence was obtained in the rest. Of 8 patients whose convalescent sera titred 32 or 64, only 2 had detectable antibody 6 months later.

6 weeks after the epidemic 150 men who had not been ill were bled and tested. 27 (18%) had antibody suggesting recent inapparent infection, giving a ratio of inapparent to apparent cases of 64:1. 30 of these negative bloods were also tested for neutralizing antibody but none was found. *C. E. Gordon Smith*

BAQUERIZO A., L. & MÁRMOL C., Fanny. Encefalitis a virus transmitidos por artrópodos. I. Variedades, vectores y posibles reservorios. II. Confirmación serológica de un caso humano tipo Venezolano. [**Virus Encephalitis Transmitted by Arthropods. I. Varieties, Vectors and Possible Reservoirs. II. Serological Confirmation of a Human Case of the Venezuelan Type**] *Rev. Ecuatoriana de Hig. y Med. Trop.* Guayaquil. 1958, July–Sept., v. 15, No. 3, 139–62. [Numerous refs.] English summary.

BERGMAN, G., LUNDSTRÖM, R. & LYSELL, L. **Rubella during Pregnancy. 2. Studies on the Dental Development in the Foetus.** *Acta Path. et Microb. Scandinavica.* 1958, v. 43, No. 1, 41–6, 3 figs.

"Histologic examination was made of the jaws of 29 foetuses; in 6 cases bilateral and in 23 unilateral. In every case there was a history of maternal rubella in the 4th to 15th week of pregnancy, counted from the last menstruation. In one foetus, anomalies of the upper incisors were observed. The mother had contracted rubella in the 9th week after the last menstruation, which can be calculated to be in the 7th week after conception, *i.e.*, when the foetus is about 23 mm in length.

"The anomaly observed, which did not seem to be in the nature of an artefact, consisted of premature eruption of the upper incisors. The possible association of this anomaly with the maternal rubella is discussed, as well as its eventual consequences for the bite."

BURNSTINE, R. C. & PAINE, R. S. **Residual Encephalopathy following Roseola Infantum.** *J. Dis. Children.* Chicago. 1959, Aug., v. 98, No. 2, 144–52, 2 figs. [12 refs.]

Roseola infantum is generally held to be a trifling disease but the authors in presenting the case histories of 6 children developing persistent neurological sequelae do not agree with this. In all except 1 the attack occurred between the ages of 12 and 16 months. All

had high temperatures with severe convulsions occurring in the febrile phase of the disease at least 2 days before the appearance of the rash. Residual effects, including hemiparesis appeared in all and at least 3 are mentally retarded and 3 epileptic. The pathological basis of the sequelae is regarded as speculative by the authors and they suggest as possibilities cerebral thrombosis, anoxia, a post-infectious encephalitis, a specific encephalitis or encephalomyelitis due to the roseola virus, the effect of prolonged convulsions, and a single and irreversible lesion of vascular or other nature. Pre-existing cerebral abnormality or some other viral exanthem such as those reported with ECHO or Coxsackie infections are also possibilities. The incidence of these sequelae in roseola infantum is uncertain but the authors state that the total reported cases with permanent sequelae now number 9. Treatment seems limited to stopping or preventing convulsions.

A. Joe

SAOUT, J. & DEMARET, G. Fausse positivité de la réaction de Waaler-Rose dans la mononucléose infectieuse. [**False Positive Waaler-Rose Reaction in Infectious Mononucleosis**] *Ann. Inst. Pasteur.* 1959, Sept., v. 97, No. 3, 428–31.

Sera from cases of infectious mononucleosis (with a positive Paul-Bunnell reaction) give false-positive Waaler-Rose reactions when the tests are carried out in the manner described by MARTIN and FLEURY (*Méd. et Lab.*, 1957, v. 7, 45). This technique requires first the absorption of the inactivated serum for one and a half hours at 37°C. with unsensitized sheep cells, followed by the reaction between the absorbed serum and sheep cells sensitized with a haemolytic rabbit antiserum. The absorption under these conditions does not render the Paul-Bunnell reaction negative, although the titre may be reduced by one or two dilutions. It seems that the remaining infectious mononucleosis heteroagglutinins agglutinate the Waaler-Rose sensitized sheep cells and this will not be recognized as fallacious unless in addition a control reaction with unsensitized sheep cells is set up. When agglutination in the control indicates that the agglutination of the sensitized cells does not have the Waaler-Rose specificity, the Paul-Bunnell test should be carried out.

Sheep cell antigens are responsible for these misleading reactions. When human group-O red cells sensitized with rabbit antihuman serum are substituted, the infectious mononucleosis sera do not give false positive reactions.

J. M. Stark

PIROSKY, I., ZUCCARINI, J., MOLINELLI, E. A., DI PIETRO, A., BARRERA ORO, J. G., MARTINI, P. & COPELLO, A. R., with the technical assistance of B. FERREYRA *et al.* Virosis hemorrágica del Noroeste Bonaerense (endemo-epidémica, febril, enantemática y leucopénica). [**Haemorrhagic Virosis of North-West Buenos Aires**] 197 pp., illustrated. English summary. 1959. Buenos Aires: Instituto Nacional de Microbiologia, Avenida Vélez Sársfield 563.

This is a detailed report of a Committee appointed by the Argentine Government to investigate outbreaks of a disease named haemorrhagic virosis of Northwest Buenos Aires. The area affected included several adjacent rural districts with a population of 268,049. The outbreak took place during the autumn and winter of 1958, starting in March, reaching a peak in June and declining first abruptly and then more gradually through August, September and October. The number of patients observed reached 260 with 48 (18%) deaths. The ages involved varied from 6 to 85 years with highest incidence among young adults (21–30 years old). Mortality rates increased with age, being of 9% and 31% among patients under and over 40 years old respectively. Of the 260 patients observed, 217 were males and 223 lived in rural areas and were mostly engaged in harvesting maize. The disease often occurred simultaneously in groups of farm labourers and there was no evidence of man-to-man transmission. Evidence is presented suggesting that the disease is an occupational hazard among maize-harvesting labourers.

An incubation period of 7 to 16 days was estimated from the study of 14 case histories. Prodromal symptoms were often protracted, consisting mainly of fever, malaise, nausea and ocular and oral enanthem. These symptoms persisted for about a week and were often accompanied by slight exanthem, micropolyadenopathy and haemorrhages from the nose and gums. Of great diagnostic value was a marked leucopenia with absence of eosinophils and relative increase of young forms of neutrophil granulocytes. Platelets were often reduced in number. The erythrocyte sedimentation rate was normal or reduced in mild cases but was often increased during the late stages of severe cases. Albumin and casts were often found in the urine. Recovery, when it took place, was uneventful and free of sequelae. Death was frequently preceded by bronchopneumonia, nephrosis and encephalopathy. Illustrative histories and detailed clinical and laboratory data of several cases are presented.

The results of 6 autopsies are described in detail. The main alteration found in all cases was a generalized capillary congestion with oedema and multiple micro- or macro-haemorrhagic lesions. Other lesions observed included hyperplasia of the reticulo-endothelial system and degenerative processes in the liver, kidneys and blood vessels.

Attempts were made to isolate the causative agent from blood, bone marrow, interstitial fluid, lymphatic gland and cerebrospinal fluid collected from a total of 20 patients. These materials were inoculated by various routes into adult or newborn Swiss mice, adult guineapigs and adult white rats. A filtrable agent pathogenic for guineapigs and mice was isolated from the blood of 3 patients. 2 strains were isolated by subcutaneous and intranasal inoculation into guineapigs and the third strain by intracerebral, subcutaneous, intraperitoneal and intranasal inoculation into adult mice. The 3 strains were propagated in newborn mice by successive intracerebral inoculation of brain suspensions. Infection of newborn mice was characterized by an incubation period of about 7 days followed by hyperexcitability, paralysis of the hind legs and positive " spin test ".

Acute and convalescent sera from 20 patients were tested by complement-fixation against normal and

infected mouse brain antigens. All sera gave negative reaction with normal brain antigens. All acute sera were negative with both antigens. 17 of the 20 convalescent sera gave complement-fixation titres against infected brain antigen ranging from 1/4 to 1/1024. A rise in virus neutralizing antibody was demonstrated by mouse protection test with paired sera from 1 patient.

A volunteer inoculated subcutaneously and intradermally with 1 ml. and 0·1 ml. respectively of a mouse brain suspension with an infectivity titre of $10^{-4 \cdot 8}$ showed typical clinical and laboratory signs of infection after an incubation period of 39 hours. Virus was isolated from blood collected on the 3rd, 6th, 7th, 10th, 11th and 12th days after inoculation. Serological evidence of infection was obtained by complement-fixation. The virus isolated was shown to be identical with a naturally occurring strain by cross mouse protection and complement-fixation tests.

The virus was not retained by a membrane filter of average pore diameter ranging from 100 to 30 mμ.

Epidemiological findings pointed to an Acarid of the Suborder Mesostigmata as a possible vector of the disease. This mite was present in great numbers in the maize plantations under study, being a common parasite of a local field mouse (*Hesperomys laucha laucha*). Newborn white mice exposed for 16 days to 2,000 acarids collected from the nest of a field mouse showed typical signs of infection. The virus responsible for this infection was propagated in newborn mice and was shown to cross-react with human strains in complement-fixation tests.

Evidence based on clinical, serological and therapeutic considerations is presented to exclude the possible aetiological role of arborviruses (groups A, B and C), rickettsiae, leptospires and fungi in the disease under study.

[A direct comparison of the agent described with known mouse pathogenic viruses would be of great interest.]

[Parts of this work were published separately in *Orientación Médica*, 1959, v. 8, 144, 156, 171, 303. A continuation of that series of papers is reviewed below.] *H. G. Pereira*

PIROSKY, I., ZUCCARINI, J., MOLINELLI, E. A., DI PIETRO, A., BARRERA ORO, J. G., MARTINI, P., MÁRTOS, Lidia & D'EMPAIRE, Matilde, with the technical assistance of T. VAZQUEZ & L. F. GUTMAN FRUGONE. Virosis hemorrágica del Noroeste Bonaerense (endemoepidémica, febril, enantemática y leucopénica). IV. Investigaciones virologicas de orden epidemiologica (primera nota). [**IV. Virological Investigations on Epidemiology**] Reprinted from *Orientación Med.* 1959, v. 8, No. 361, 708–10.

The authors report the isolation of the virus associated with haemorrhagic virosis of North-west Buenos Aires from field mice (*ratones maiceros*) of the species *Hesperomys laucha laucha*. 2 of 20 mice captured in a maize plantation situated within the epidemic area showed signs of infection after a period of 7 days in isolation from other mice. A virus was isolated from brain suspensions of the diseased mice by intracerebral inoculation into newborn white mice. Antigens prepared from infected mouse brains gave positive complement-fixation reactions with convalescent human sera from the epidemic under study. No virus was demonstrated in the brains of healthy field mice. Sera from these mice showed, however, neutralizing antibodies suggesting recovery from natural infection. [Parts I–III of this series contain information incorporated in the monograph above.] *H. G. Pereira*

Bacteriology and Immunity

The order in this section is:—**General; Bacteriostatic and Bactericidal Substances; Bacteria; Rickettsiae; Bacteriophages; Viruses; Immunity.**

Abstracts of papers relating to the bacteriology of plague, cholera, yaws, leprosy and certain other tropical bacterial diseases; of the rickettsial fevers; and of yellow fever, dengue, rabies and certain other tropical virus infections, are published in the TROPICAL DISEASES BULLETIN. *Their titles and page references are cited below in the appropriate sub-divisions of this section.*

GENERAL

NORRIS, K. P. **Infra-Red Spectroscopy and its Application to Microbiology.** *J. Hygiene.* 1959, Sept., v. 57, No. 3, 326–45, 2 figs. [Numerous refs.]

Infra-red (I-R) spectroscopy in the region 1–16μ may be applied to the characterization of bacterial suspensions, virus preparations or cellular extracts. The interference of the absorption bands of water may be avoided by comparing the spectrum in D_2O or more commonly by using cell suspensions dried on AgCl films or freeze dried organisms mixed with KBr or KCl and pressed into transparent disks.

Differentiation (rather than identification) of whole cells grown under standardized conditions may be

achieved by I-R spectroscopy. Dense common bands obscure fine detail but resolution is possible by a variety of techniques. Numerous examples of successful differentiation are given. Virus characterization is difficult owing to contaminating host material. Cellular extracts are of greater value in differentiating organisms, *e.g.* the lipid fractions of mycobacteria. The capsular substances of pneumococci have been typed by I-R spectroscopy and results parallel to the immunological ones obtained. Enzymes and reagents with known action may be used to remove substances with dense interfering bands. The appearance and disappearance of bacterial metabolites may be followed, *e.g.* the release of Ca dipicolinate into the medium on spore germination and the accumulation of glycogen in coliforms and β-hydroxybutyric acid in *B. cereus*.

The characterization of cellular extracts is the most precise application and the identification of the rarer sugar components of polysaccharides and their mode of linkage, the analysis of new antibiotics and the finding of rubber among the products of certain fungi have depended upon this method.

[This is a good introduction to a technique of great promise if not misused.] *Stewart Fletcher*

KOSUNEN, T. **Acute Experimental Infections in Mice. Quantitative Microbial Studies.** *Ann. Med. Exper. et Biol. Fenniae.* Helsinki. 1959, v. 37, Suppl. 2, 88 pp., 42 figs. [135 refs.]

The course of an experimental staphylococcal infection was studied by enumeration of viable organisms in the various organs of mice killed at intervals over a period of 2 to 4 weeks. The method used was similar to that of SMITH and DUBOS [this *Bulletin*, 1956, v. 31, 673]. A dose of organisms approximately one-tenth of the LD50 injected intravenously resulted in a rise in the numbers of bacteria in the kidneys during the first 5 days after infection followed by a steady decline; in the spleen and liver decreasing numbers were found from the first day after challenge. These results are in agreement with those of Smith and Dubos.

The effect of treating with chloroform and carbon tetrachloride mice injected with staphylococci was examined. The bacterial count in the kidneys, liver and spleen of animals thus treated was higher than in the controls. In contrast the count was lower in mice which were injected either with penicillin or ristocetin.

The experiments gave the same results with a strain of *Streptococcus faecalis*.

A strain of *Candida albicans* given intravenously in a dose of 4×10^3 (LD50 = $9{\cdot}9 \times 10^4$) caused an infection characterized by an initial rise in the viable count or organisms in the kidneys. The antifungal effects of nystatin, amphotericin B, and candidin were demonstrated by the reduction of the number of microbes in mice receiving these substances.

[The author has demonstrated that by this method results can be accurately reproduced, but it seems doubtful whether the problems examined warranted a procedure more complex than a simple determination of LD50.] *Douglas D. Smith*

DEARMON, I. A., Jr. & LINCOLN, R. E. **Number of Animals required in the Bio-Assay of Pathogens.** *J. Bacteriology.* 1959, Nov., v. 78, No. 5, 651–7. [17 refs.]

PETERSDORF, R. G. & BENNETT, I. L., Jr. **The Experimental Approach to the Mechanism of Fever.** *Arch. Intern. Med.* 1959, June, v. 103, No. 6, 991–1001, 10 figs. [36 refs.]

Although certain bacteria and viruses have intrinsic pyrogenic properties, fever, in general may be regarded as a host response to tissue injury. In order to test this hypothesis the ability of injured cells, extracts of damaged tissue and other substances to cause fever has been examined.

Lipopolysaccharide endotoxins, prepared from Gram-negative bacilli, or heat killed suspensions of whole bacteria will produce high fever when injected intravenously into man or animals, often after a very short interval. After the abrupt initial rise in temperature there is a transient fall followed by a secondary peak the biphasic response, which is characteristic of an endotoxin fever. This effect is associated with a very pronounced but transient leucopenia which is assumed to be the result of injury to the circulating leucocytes by the bacterial toxins; the leucocytes leave the circulation and are sequestrated in the capillary beds of various organs. After an interval of 1–2 hours the leucopenia is replaced by a fairly marked leucocytosis. Repeated injection of endotoxin establishes a tolerance but this can be broken down by the administration of reticulo-endothelial "blocking" agents such as colloidal thorium dioxide or trypan blue. Tolerance is, however, a relative dose-dependent phenomenon and animals rendered refractory will usually respond to suitably increased doses of endotoxin.

In addition to the direct fever-producing endotoxin a secondary substance appears in the serum of animals about an hour after they have received endotoxin: this is known as endogenous pyrogen which, passed to either normal or tolerant animals, produces a brief monophasic fever. It would therefore appear that endotoxin fever is mediated by 2 distinct mechanisms: (1) A direct action of the toxin on the brain. The appearance of a pyrogenic substance, indistinguishable from bacterial endotoxin, in the cerebrospinal fluid within 15 minutes of the intravenous injection of endotoxin would support this view. (2) The action of the endotoxin on the cells releases the endogenous pyrogen which can also produce fever.

There is some indication, however, that fever associated with the endotoxins of Gram-negative bacteria can be largely independent of the endogenous pyrogen mechanism, whereas other fevers, such as those associated with pneumococcal infections or those produced by the injection of influenza viruses, seem to be related solely to the endogenous pyrogen mechanism. The whole subject is extremely complicated and there are many gaps in our knowledge of the problem. Much further research is required before the complicated processes involved can be properly understood. *H. J. Bensted*

MACCACARO, G. A.; COLOMBO, C.; DI NARDO, A.; TURRI, M.; DETTORI, R. Studi sulle fimbrie batteriche. I. Lo studio genetico delle fimbrie [**Study of Bacterial Fimbriae. I. Genetic Study**] [MACCACARO, COLOMBO & DI NARDO]. *Giorn. di Microbiol.* Naples. 1959, Jan.–Mar., v. 7, No. 1, 1–20, 1 fig. [19 refs.] English summary. II. Osservazioni microelettroforetiche [**II. Microelectrophoretic Observations**] [MACCACARO & TURRI]. *Ibid.*, 21–36, 4 figs. [11 refs.] English summary. III. L'effetto delle fimbrie sulla respirazione di colture in accrescimento [**III. Effect of Fimbriae on the Respiration of Growing Cultures**] [DETTORI & MACCACARO]. *Ibid.*, 37–51, 8 figs. English summary. IV. Metabolismo ossidativo e fermentativo in cellule fimbriate e sfimbriate [**IV. Oxidative and Fermentative Metabolism in Fimbriated and Non-Fimbriated Cells**] [MACCACARO & DETTORI]. *Ibid.*, 52–68, 5 figs. English summary. V. Sferoplasti fimbriati [**V. Fimbriated Spheroplats**] [MACCACARO & TURRI]. *Ibid.*, 69–80, 1 fig. on pl. [13 refs.] English summary.

WIDRA, A. **Metachromatic Granules of Microorganisms.** *J. Bacteriology.* 1959, Nov., v. 78, No. 5, 664–70, 9 figs. [41 refs.]

O'LEARY, W. M. **Involvement of Methionine in Bacterial Lipid Synthesis.** *J. Bacteriology.* 1959, Nov., v. 78, No. 5, 709–13. [20 refs.]

STEWART, J. E. & KALLIO, R. E. **Bacterial Hydrocarbon Oxidation. II. Ester Formation from Alkanes.** *J. Bacteriology.* 1959, Nov., v. 78, No. 5, 726–30, 5 figs. [16 refs.]

GIESBRECHT, P. Elektronenmikroskopische Darstellung verschiedener chemischer und physikalisch-chemischer Reaktionen an den DNS-Strukturen eines Chromosoms. (Die Bakterienzelle als "biologisches Reagenzglas".) [**Electronmicroscopical Demonstration of Different Chemical and Physico-Chemical Reactions in the DNS Structures of a Chromosome (The Bacteria Cell as "Biologic Test Tube")**] *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 176, Nos. 7/8, 413–41, 16 figs. (14 on 5 pls.) [43 refs.]

BACTERIOSTATIC AND BACTERICIDAL SUBSTANCES; DISINFECTION

The order in this section is:—**General; Antibiotics; Colicines; Other Therapeutic Substances; Disinfection.**

FINLAND, M., JONES, W. F., Jr. & BARNES, Mildred W. **Occurrence of Serious Bacterial Infections since Introduction of Antibacterial Agents.** [Report to the Council on Drugs (KAUTZ, H. D., Secretary).] *J. Amer. Med. Ass.* 1959, Aug. 29, v. 170, No. 18, 2188–97, 16 figs. [Numerous refs.]

This is a very important paper, clearly revealing highly disturbing developments. What the authors have done is to compile data about certain acute infections in the Boston City Hospital for the years 1935 (*i.e.*, before the introduction of anti-bacterial chemotherapy), 1941 (when sulphonamides were in full general use), and 1947, 1951, 1953, 1955 and 1957 as reflecting the use of increasing numbers of antibiotics. They present in a series of diagrams the frequency with which (1) bacteriaemia, (2) meningitis, (3) empyema, was due to different species of (a) Gram-positive cocci, (b) Gram-negative bacilli. Among the former organisms, pneumococci and haemolytic streptococci, particularly the latter, have diminished in importance, while staphylococcal infections of the blood and meninges have increased greatly in numbers. Among Gram-negative infections, there has been an enormous increase in the frequency of bacteriaemia due to *Proteus, Klebsiella aerogenes* and *Pseudomonas pyocyanea*, and of meningitis due to similar organisms. Findings for several other species in both groups are included.

Mortality trends are also analysed: the number of deaths from bacteriaemia diminished until 1947, but has risen steadily since and in 1957 was 50% above the 1935 level, the great majority being due to staphylococci or one of the 3 Gram-negative species named above. The case mortality of bacteriaemia, which fell in the early part of the period, has risen in the latter part of it, particularly in patients in the higher age groups. These are only the broad outlines of the results: the findings include many interesting points of detail. The authors present these facts without trying to interpret them, except by pointing out that the organisms now killing patients are in general antibiotic-resistant, and that antibiotics alter the general body flora apparently to the advantage of such species. [That death from septicaemia should now be commoner than in 1935 is certainly a portentous fact, calling both for full elucidation and for energetic action.]

L. P. Garrod

KIRSHBAUM, A. & ARRET, B. **Outline of Details for Assaying the Commonly used Antibiotics.** *Antibiotics & Chemotherapy.* New York. 1959, Oct., v. 9, No. 10, 613–17.

"A table has been presented listing details required for 36 different bioassay methods for antibiotics; a total of 17 of the most commonly used antibiotics are included in these methods. If the analyst is already

familiar with routine bioassay procedures the table gives him all the information required for performance of the assays."

BJÖRNBERG, A. **Hallucinatory Reactions in association with Oral Administration of Probenecid-Penicillin.** *Acta Med. Scandinavica.* 1959, Oct. 28, v. 165, No. 3, 207–10. [15 refs.]

"Hallucinatory reactions to perorally administered probenecid-penicillin were observed in three patients who received this treatment for epidemic influenza acute pharyngitis or erysipelas. The reactions showed some resemblance to the acute psychotic reactions which have been seen in association with injection of procaine penicillin."

MANTEN, A. & DE NOOY, J. A. **Some Further Observations on Synergism between Chloramphenicol and Polymyxin B in relation to *Salmonella* Bacteria.** *Antonie van Leeuwenhoek: J. Microbiol. & Serol.* 1959, v. 25, No. 2, 183–7, 1 fig. [13 refs.]

Salmonella typhi-murium was rapidly killed by 1 μgm./ml. of polymyxin, but 0·3 μgm./ml. did little more than inhibit growth, as did chloramphenicol in concentrations of 5–100 μgm./ml. When both antibiotics were added to a culture in concentrations which were individually only inhibitory (0·3 and 10 μgm./ml. respectively), a rapid bactericidal effect was obtained. The authors hope to exploit this synergic effect for the treatment of *Salmonella* carriers, giving both antibiotics orally, and results already obtained, of which no details are given, are said to "justify some optimism". *L. P. Garrod*

IVÁNOVICS, G., NAGY, E. & ALFÖLDI, L. **Megacinogeny: Inducible Synthesis of a New Immunospecific Substance.** *Acta Microbiologica.* Budapest. 1959, v. 6, No. 2, 161–9, 3 figs. [17 refs.]

By a process of adsorption and elution, megacin was obtained in concentrated form from culture of *Bacillus megaterium* in which its formation had been induced by ultraviolet irradiation. It was found to be highly unstable to heat in only slightly alkaline medium, and more stable in acid. Pepsin and chymotrypsin inactivated it rapidly: purified trypsin did not (at *p*H 7·17). Rabbits were immunized with it, and their serum precipitated megacin, neutralized its bactericidal action, and fixed complement with it, but not with an extract of unirradiated culture. It is concluded that megacin is a specific protein, the induced formation of which is an example of lethal biosynthesis. *L. P. Garrod*

IVÁNOVICS, G., ALFÖLDI, L. & NAGY, E. **Mode of Action of Megacin.** *J. General Microbiol.* 1959, Aug., v. 21, No. 1, 51–60, 5 figs. [17 refs.]

Megacin was obtained from culture of *Bacillus megaterium* in a defined medium, subjected to ultraviolet irradiation, and concentrated by a process of adsorption and elution. Its bactericidal action was found to be highly temperature-dependent, none being exerted at 0°C. When added to a young culture of *B. megaterium* in a liquid medium it caused a decrease in turbidity; this was not due to lysis, but to the escape of intracellular material: the "empty" cells were observed (and counted) microscopically and the liberated material was demonstrated in the supernatants. When the medium contained lactose, β-galactosidase was liberated. Lysozyme-prepared protoplasts of *B. megaterium* and of a megacin-sensitive coccus were converted to ghosts by megacin, but there was no effect on protoplasts of species insensitive to it. Megacin is believed to act on the osmotic barrier of sensitive cells, either directly as an enzyme or by activating an intrinsic autolytic enzyme. *L. P. Garrod*

HAMON, Y. & PÉRON, Yvonne. Emploi des antibiotiques bactéricides pour l'obtention des préparations stériles de bactériocines. [**Use of Bactericidal Antibiotics to obtain Sterile Preparations of Bacteriocins**] *Ann. Inst. Pasteur.* 1959, Oct., v. 97, No. 4, 518–25. [17 refs.]

The "bacteriocins" with which this paper is chiefly concerned are colicines. FREDERICQ [this *Bulletin*, 1950, v. 25, 1062] recommended that *Escherichia coli* forming these substances should be killed with chloroform, but some of them are destroyed by this treatment. These authors use streptomycin in a concentration of 200 or 500 μgm./ml. (polymyxin is an alternative) and proceed then to determine the amount of colicine formed with an indicator strain resistant to the antibiotic used. It is mentioned that the same method can be used for obtaining bacteriophage free of bacteria.

L. P. Garrod

PAPAVASSILIOU, J. **Production of Colicines in Simmons's Citrate Agar.** [Correspondence.] *Nature.* 1959, Oct. 24, v. 184, Suppl. No. 17, 1339–40.

RENTCHNICK, P. & LAGIER, J. Un nouveau sulfamidé-retard: la sulfadimethoxine (Madribon) [**A New Long-Acting Sulphonamide, Sulphadimethoxine (Madribon)**] *Schweiz. med. Woch.* 1959, Aug. 22, v. 89, No. 34, 894–8, 7 figs.

This is a mainly pharmacological study of 2,4-dimethoxy-6-sulphanilamido-1,3-diazine (sulphadimethoxine; "Madribon"). Blood level curves after single large doses showed a slightly less prolonged effect than that produced by sulphamethoxy-pyridazine, but a higher and more sustained blood level than that found for sulphaphenylpyrazole, in each case in the first-named author's own previous studies. Further studies of the levels obtained by repeated doses lead to the recommendation that a loading dose of 2 gm. be followed by 0·5 gm. daily. The degree of acetylation in both blood and urine was found to be low. A few observations showed satisfactory levels in bile and pleural effusion, but very low levels relative to those in the blood in cerebrospinal fluid. It is also briefly reported that generally satisfactory therapeutic result

were obtained in infections (of unspecified aetiology) of the throat, lungs and urinary tract (40 patients in all). The only side effect observed was severe headache in 1 patient. *L. P. Garrod*

HARMSEN, H., OSTERTAG, H. & PREUSS, H. Neue bakterizide Ampholytseifen als einzeitig und schnell wirkende Händedesinfektionsmittel. [**New Bactericidal Ampholyte Soaps as Immediate and Rapidly Acting Hand Disinfectants**] *Deut. med. Woch.* 1959, Sept. 4, v. 84, No. 36, 1590–95, 1664, 2 figs.

This paper is in praise of Tegolan, the active constituents of which are dodecyl-1,3-aminopropyl-β-aminobutyric acid and dodecyl-1,3-oxypropyl-β-aminobutyric acid in an emulsified form. The preparation has a low surface tension, excellent cleansing properties and no irritant effect. Particulars are given of its inhibitory and bactericidal action on 4 species of bacteria. Tests of disinfecting action on skin were made on hands artificially contaminated with *Escherichia coli*, and on others in their natural state, the effect being judged by reduction in total count. [The reader is referred to other papers for descriptions of the methods used: without reference to these, and in the absence of certain controls and of any adequate comparison with other skin disinfectants, the results are difficult to evaluate.] It was also found that a persistent action is obtained, the bacterial content of rubber gloves worn for 1 hour after disinfection of the hand being low. The preparation has been perfectly tolerated during extensive use. *L. P. Garrod*

BACTERIA

The order in this section is:—**Cocci; Donovania; Mycobacterium; Corynebacterium; Haemophilus; Pseudomonas; Pfeifferella; Enteric organisms; Brucella; Pasteurella; Bacillus; Anaerobes; Spirochaetes; Erysipelothrix; Listeria; Others.**

VOGELSANG, T. M. & HAALAND, H. **Studies of Pathogenic Staphylococci in the Upper Respiratory Tract of Members of Hospital Staff. 1. Carrier Rate of Pathogenic Staphylococci.** *Acta Path. et Microb. Scandinavica.* 1959, v. 45, No. 1, 67–76. [21 refs.] **2. Antibiogram Determinations.** *Ibid.*, 77–94. [16 refs.] **3. Bacteriophage Typing.** *Ibid.*, No. 3, 281–94. [15 refs.] **4. Phage Groups and Phage Patterns of Antibiotic Resistant Staphylococcal Strains.** *Ibid.*, No. 3, 295–308. [15 refs.]

1. Staphylococci were sought in the throat and nose of 845 members of clinical and non-clinical personnel in hospital in Bergen. Coagulase positive strains isolated from either site ranged from 67·2% in non-clinical departments to 81·2% in the neurological department. 2·3% of coagulase positive strains failed to ferment mannitol and 13·5% produced no haemolysis on human blood agar plates. The authors stress the importance of throat and nose cultures since 23·3% of strains were isolated from the throat only and 16·6% from nose only.

2. The coagulase-positive staphylococci (995 strains) described in the previous study were investigated for resistance by the disk method to 10 drugs including the common antibiotics. 37·1% of all strains were resistant to penicillin and resistance in the departmental groups varied from 13·8% in 1st year nurses to 50% in medical departments. If relatively penicillin-resistant strains are included 61·3% of strains from the throat and 67% from the nose were resistant. 1/5 of strains were resistant to streptomycin, 1/10 resistant to sulphathiazole and only a few were resistant to tetracyclines, oleandomycin, chloramphenicol and novobiocin. No strains were resistant to erythromycin or nitrofurantoin. 21·4% of strains were resistant to more than one antibiotic.

3. The staphylococcal strains from the previous study were bacteriophage typed and classified according to phage group and hospital department to which the carrier of the strain belonged. 11·2% of strains were not typable. Strains of group III predominated in all departments (38·2% of all strains). Group II strains accounted for 12·7% and group I, 19·9%. There was no predominance of individual phage types in the clinical departments compared with the non-clinical departments.

4. The results of the antibiotic resistance and phage typing of the strains in the previous studies are compared. 53·1% of the penicillin-resistant strains belonged to group III. Similar percentage of strains came from clinical and non-clinical departments. 60·9% of streptomycin-resistant strains belonged to group III; 93% of them came from people working in clinical departments. Of the 28 tetracycline-resistant strains 26 were from clinical personnel and 23 were of group III. 101/151 strains resistant to more than one antibiotic belonged to group III and 38 to group I. Although the majority of resistant strains still belong to group III there is an increase of resistant group I strains compared with previous studies by these workers. [VOGELSANG, this *Bulletin*, 1959, v. 34, 169.]

M. Patricia Jevons

PAPAVASSILIOU, J. & OBIGER, G. Über das Vorkommen und das Phagenbild von Koagulase-positiven Staphylokokken in Milch. [**Occurrence of Coagulase-Positive Staphylococci in Milk and their Phage Types**] *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 175, Nos. 7/8, 505–19. [35 refs.]

Some 500 samples of milk (including 100 samples of pasteurized milk) and a large number of swabs from milk pails and equipment, and from milk handlers, were examined for *Staphylococcus aureus*, which was

found in about 34% of the raw milk samples but in only 1 of the pasteurized. The staphylococci were submitted to a number of the conventional tests and for the most part the results conformed with general experience. Surprisingly, however, 47% of the coagulase-negative strains hydrolysed colaminphenolphthalein phosphate. 84 of the 100 strains tested were typable by phage; 57 fell into group III and 24 were lysed by phage 42D as well as 1 or more group III phages; only 2 were lysed by 42D alone.

Only 7 of the 110 strains reacted with Oeding's *ac* factor serum, which had been thought to be species-specific. *R. E. O. Williams*

Bortolini, G. & Stocchi, F. Alcune prove di inibizione della coagulasi stafilococcica con sostanze anticoagulanti. [**A Study of the Inhibitory Action of Some Anticoagulants on Staphylocoagulase**] *Arch. Ital. Sci. Med. Trop. e Parassit.* 1959, July, v. 40, No. 7, 373–88. [Numerous refs.] English summary.

The purpose of this work was to study the point at which certain anticoagulants with a known way of action would affect the coagulative activity of staphylococci and thus to learn its mechanism. 10 strains which on clinical and biochemical grounds could be certainly considered to be pathogenic were used. The anticoagulants were (1) a solution of heparin containing 5,000 units per ml., (2) a solution of phenylindanedione, (3) a solution of trypan blue, and (4) a solution of Congo red. The staphylococci were cultivated on heart and brain infusion broth. Tests were carried out in 0·5 ml. Bacto coagulase plasma, made up in saline before use at a concentration of 30 mgm. of the dried plasma per ml. In 1 set of 6 tubes 0·5 ml. of heparin solutions were made at doubling up final strengths from 75 to 2,500 units. A second set contained 0·5 ml. of phenylindanedione in final strengths of 0·1 to 3·2 mgm. per ml. In a third series 0·5 ml. of Congo red solution was used at final dilutions ranging from 1/50 to 1/1,600. A fourth series contained similar concentrations of trypan blue. 2 drops of the 24-hour staphylococcal broth cultures were added. Readings were taken after 30 and 60 minutes, and 2, 12 and 24 hours. Coagulation in control tubes occurred regularly within 2 hours. Heparin delayed but did not prevent coagulation, which was complete at all dilutions after 24 hours. The delaying action was greatest in the presence of the greater concentrations. Phenylindanedione acted similarly. Coagulation did not take place in the presence of the dyes even after 48 hours.

The writers suggest that heparin which acts on thrombin, prothrombin and thromboplastin, and phenylindanedione which acts on factor VII and on prothrombin could affect free coagulase but cannot act on coagulase in the bacterial cell, which uniting with a plasma factor can form a thrombin-like compound which will bring about coagulation, although this may be delayed. Dyes, on the contrary, impede coagulation, acting on fibrinogen. *E. Agius*

Mangalo, R. & Raynaud, M. Étude cinétique de l'hémolyse des globules rouges de lapin par la toxine staphylococcique alpha. [**Kinetic Study of the Haemolysis of Rabbit Erythrocytes by α Staphylococcal Toxin**] *Ann. Inst. Pasteur.* 1959, Aug., v. 97, No. 2, 188–97, 7 figs. & 1 pl.

The progress of haemolysis of rabbit red cells under the influence of staphylococcus α-toxin was followed by observing the transmission of light of wavelength 650 mμ in a spectrophotometer. The T_{50}, the time to lyse 50% of the red cells, was derived from a probit transformation of the percentage lysed plotted against log time. The optimum temperature was 35°C. with a steep decline in activity above 40°C., thought to be due to destruction of the toxin or the receptors on the red cells. There was a straight-line relation between T_{50} and log concentration of toxin. The optimum *p*H was 5·2 in acetate buffer. Variation in the concentration of red cells had little effect. *R. E. O. Williams*

Bergdoll, M. S., Surgalla, M. J. & Dack, G. M. **Staphylococcal Enterotoxin. Identification of a Specific Precipitating Antibody with Enterotoxin-Neutralizing Property.** *J. Immunology.* 1959, Sept., v. 83, No. 3, 334–8, 2 figs. [15 refs.]

Partially purified enterotoxin from a strain of *Staph. aureus* S-6 was used to prepare antisera in rabbits and monkeys by various techniques. Gamma globulins were separated from the rabbit antisera.

Gel diffusion tests showed that the monkey antiserum contained 3 predominant antibodies; the antigen reacting with one of these was shown by monkey feeding tests to contain the enterotoxin. It was purified and used to immunize rabbits. After immunization the serum contained only 1 antibody to S.6 as judged by gel-diffusion and neutralization tests.

The equivalence ratios of the antigen-antibody system obtained by the Oudin test was 2–3 times as high as that determined by neutralization tests.

This monovalent serum, however, did not cross-react with partially purified enterotoxin from a powerful enterotoxin-producing strain 196E although it reacted with 13 other enterotoxin-producing strains in the authors' collection. Possible differences in enterotoxin are discussed. *M. Patricia Jevons*

Visco, G. & Salvi, L. Studi sul sinergismo tra virus influenzale e germi. Nota IV. Modalità di sviluppo del *Myxovirus influenzae A* e dello *Staphylococcus pyogenes aureus* nell'infezione mista della cavità allantoidea dell'embrione di pollo. [**Synergism between the Influenza Virus and Bacteria. IV. The Development of Myxovirus Influenza A and of *Staphylococcus pyogenes aureus* in Mixed Infection in the Allantoic Cavity of the Chicken Embryo**] *Riv. Istituto Sieroterap. Ital.* 1959, July–Aug., v. 34, No. 4, 251–62, 1 graph. [21 refs.] English summary (7 lines).

The object of this investigation was to study the growth curves of the influenza virus and of staphylococcus inoculated simultaneously in the allantoic cavity

of the chicken embryo and to compare them with similar curves of the micro-organisms inoculated separately. After tests to find the most suitable size of inocula, 68 embryos were inoculated with 10^5 staphylococci in 0·2 ml. of diluent, 40 with the influenza virus (0·10 ml. of allantoic fluid diluted to 10^{-5} and 0·10 ml. of broth), and 68 received an inoculum of 0·2 ml. containing the same quantities of staphylococci and influenza virus as were used separately. Bacterial counts were made by the normal pour plate method, viral estimation by titrating haemagglutinins by Hirst's method as modified by Salk.

There was no noticeable difference between the growth curve of *Staphylococcus aureus* growing by itself and in the presence of influenza A virus. The haemagglutinin titres of the virus were noticeably higher in the case of mixed infection, and the viral haemagglutinins appeared earlier. The difference between the growth curves for the virus under the two conditions was statistically significant, which was not the case for the difference between the staphylococcal growth curves. This phenomenon could be the result of a non-specific potentiating process analogous to the effect of inorganic adjuvants in viral vaccines. The authors' findings suggest that if there is a potentiating effect on the bacteria by the tissues of the host necrotized by the viral infection this does not take the form of an increased bacterial multiplication.

E. Agius

Oeding, P. & Östervold, B. **The Stability of Oxford *Staphylococcus* H and other Penicillin Reference Strains.** *Acta Path. et Microb. Scandinavica.* 1959, v. 46, No. 2, 149–55.

Strains of staphylococci kept as reference strains for penicillin sensitivity determinations were collected and examined. 19 strains were obtained from various laboratories where they had been kept under a variety of conditions. 11 were designated Oxford staphylococus, 4 designated 209P and 1, ATCC 6538P, was known to be identical with 209P [this *Bulletin*, 1954, v. 29, 666].

All strains were sensitive to 0·04 unit/ml. penicillin. Only 1 of the Oxford strains but all other strains were pigmented. There were minor differences in biochemical activity but wide differences in α-haemolysin production. Although all Oxford strains except 1 were similar their characteristics differed from the original characteristics of this strain.

Phage typing gave identical results on all Oxford strains except 1; serological typing of these strains differed only in a minor degree probably owing to the ropy consistency of old laboratory cultures. All 209P and 6538P strains were identical by phage and serological typing and 2 of the other strains may have been strains of this type but showed minor differences.

The authors conclude that, while minor cultural and biochemical changes develop on laboratory storage, the penicillin sensitivity, phage type and serological type are stable under these conditions. *M. Patricia Jevons*

Medical J. Australia. 1959, Sept. 12, v. 2, No. 11, 355–7. **A Survey of the Penicillin Sensitivity of *Staphylococcus aureus* in General Practice.**

This paper reports the findings of an investigation carried out by the Research Committee of the New South Wales Faculty of the Australian College of General Practitioners. In 337 reports on clinical material from 26 practices, the staphylococcus cultivated was found to be penicillin-resistant in 76·6%. These, however, were not consecutive cases, but "the more severe infections or those less responsive to the initial treatment". In the practice in Sydney of the recorder of this investigation and apparently author of this report, 102 consecutive cases of staphylococcal infection, regardless of their severity, were examined, and the frequency of penicillin resistance was 60·2%. [Even this is a very high proportion for a general population.] That for Sydney hospitals is given as 74·4%. *L. P. Garrod*

Finland, M., Jones, W. F., Jr. & Bennett, I. L., Jr. **Antibiotic Susceptibility and Phage Types of Pathogenic Staphylococci. A Study of Two Hundred Ten Strains isolated at Boston City Hospital in 1955.** *Arch. Intern. Med.* 1959, Sept., v. 104, No. 3, 365–77, 3 figs. [10 refs.]

The antibiotic sensitivity was determined of 210 strains of *Staph. aureus* isolated during a 2-month period in 1955 in the Boston City Hospital. 18 antibiotics were tested, 10 of which had not been used, or had rarely been used, in the hospital and strains were uniformly sensitive to those agents which were unrelated to antibiotics in common use. There was a wide spread between minimum and maximum inhibitory concentrations of all common antibiotics except chloramphenicol. 80% of the strains were not inhibited by 100 mgm./ml. penicillin. Strains resistant to oleandomycin were all resistant to erythromycin but the converse was not true.

148 strains were isolated from patients who had had antibiotic therapy (usually multiple) prior to isolation of the strains. These strains were compared with 62 strains isolated from patients who had had no antibiotics, for sensitivity to penicillin, streptomycin, tetracycline and erythromycin. Pretreatment strains included a higher number of susceptible strains, and strains from autopsies contained a higher proportion of tetracycline-resistant strains than those isolated in life.

Of the 180 strains which were phage-typed 61 were type 52/42B/81, about one-quarter were non-typable and the remaining 71 strains belonged to 36 different types. The distribution of types was similar in treated and untreated patients. The majority of 52/42B/81 strains were resistant to penicillin and streptomycin and 84% were resistant to tetracyclines. The hospital practice of restricted use of erythromycin may have accounted for the susceptibility of the epidemic type to the drug. The non-typable strains contained the highest proportion of erythromycin-resistant strains.

More than 1 strain was isolated from 19 patients and the majority of strains of different antibiotic pattern from the same patient were shown by phage typing to

be different from the original although increase in resistance of a strain during treatment was demonstrated in 3 cases. *M. Patricia Jevons*

COLLARD, P. **The Incidence of Antibiotic Resistant Staphylococci Isolated at Ibadan 1956–1958.** *West African Med. J.* 1959, Oct., v. 8 (n.s.), No. 5, 197–202, 7 figs.

The percentage of strains of *Staphylococcus aureus* encountered in Ibadan, Nigeria, from inpatients and outpatients in the years 1956–58 are compared for their resistance to penicillin, streptomycin, aureomycin and chloramphenicol [total numbers are not given].

The percentage of fully sensitive strains from outpatients has fallen from 77% in the first quarter of 1956 to 8% in the last quarter of 1958, at which time 19% of strains from inpatients were resistant to all 4 antibiotics. The percentage of strains resistant to all 4 antibiotics has risen but this rise is least marked with chloramphenicol.

The author attributes this alarming rise in the number of antibiotic-resistant strains to the sale of antibiotics without prescription. *M. Patricia Jevons*

WAISBREN, B. A. & STRELITZER, C. L. **Antibiotic Sensitivities of Staphylococci isolated before and after Patients were given Antibiotics.** *Amer. J. Med. Sci.* 1959, Aug., v. 238, No. 2, 202–10, 1 fig. [19 refs.]

This paper analyses findings in 50 patients with staphylococcal infections, consisting of "chronic bacteremias, chronic osteomyelitis and wound infections". They were treated with various antibiotics, and cultures obtained before and after 152 courses of treatment were compared. In 53 there was no change in sensitivity, while 57 were more resistant and 42 more sensitive. Very similar figures were obtained when the cultures were compared in sensitivity to an antibiotic other than that administered. These sets of findings are presented graphically, taking into account the degree of difference in sensitivity in the tube dilution tests, and the 2 curves are almost superimposed. Full details for each of 9 antibiotics and nitrofurantoin are given in a double-page table: changes to greater resistance were much more frequent than to increased sensitivity only after the administration of erythromycin, when the degree of increased resistance was usually large, and after neomycin, when it was small. Greater resistance was somewhat commoner after the administration of 2 antibiotics together than after only 1.

The authors speculate on the meaning of these findings: replacement with another strain is considered as one possibility, and was found to have occurred in the only 4 instances in which phage typing was applied.

[Nothing is said about length of treatment or the time relation of the second culture to the end of the course, and no absolute values for sensitivity are given. The large increases in sensitivity are very puzzling: when the after-treatment culture is found more sensitive than the original to the extent of 7, 8 or even 10 tubes—*i.e.* by up to 1,000-fold—one wonders why that antibiotic was given at all.] *L. P. Garrod*

RHEUBEN, J. A., BULLEN, M. & THOMSON, E. F. **Development of Resistance to Antibiotics by Staphylococci: a Comparative Study using Tetracycline and Oleandomycin separately and in combination.** *Med. J. Australia.* 1959, Sept. 12, v. 2, No. 11, 353–5, 1 fig.

This paper reports the results of trying to habituate staphylococci to tetracycline, oleandomycin and Synermycin (a 2:1 mixture of these antibiotics) by the usual procedure. There were 2 series of tests: in the first, some of the 12 strains used were initially resistant to one of the antibiotics [there is an error in Table 1, strain 4 being described as resistant to tetracycline in one place and sensitive in another]. All became resistant to each single antibiotic and 3, although more slowly, to synermycin. It was thought advisable to repeat this, and 13 further strains were used, none fully but 2 partially resistant to one of the antibiotics. All became resistant (not inhibited by 100 μgm./ml.) to oleandomycin in 7 transfers or less: 3 became resistant to this degree and 9 in lesser degrees to tetracycline in 19 transfers or less, but in 19 transfers of each strain resistance to synermycin never increased more than 16-fold, the highest inhibitory concentration reached being 5 μgm./ml. In one strain it actually diminished by 4-fold. Substitution was excluded by redetermining the phage type at the end of the test. *L. P. Garrod*

WEISER, R. S. & BOATMAN, E. S. **The Growth of *Streptococcus pyogenes* within Agar Disks implanted in the Peritoneum of the Rabbit.** *J. Infect. Dis.* 1959, Sept.–Oct., v. 105, No. 2, 168–79, 4 figs. [11 refs.]

The authors failed to produce rheumatic disease in rabbits by the introduction into the peritoneum of Group A streptococci in agar disks in glass cylinders [WERNER *et al.*, this *Bulletin*, 1954, v. 29, 1176].

The strains used had been isolated from patients with rheumatic fever and were said to be pathogenic for rabbits but their antigenic state at the time of study is not given. A strain of type 12 was mainly used. Disks were removed at varying intervals and replaced by fresh disks. In some animals this was repeated 8 times.

The appearance of the disks is described. Growth of organisms was restricted to a narrow zone beneath the outer sterilized area. It was visible at 2 days, maximal at 4 days and thereafter there was a rapid decline in the number of viable organisms. It is suggested that this decline was due to the accumulation of Tillet factor in the disk [TILLETT, this *Bulletin*, 1937, v. 12, 300]. [This factor was tested for by determining the extent to which a saline extract of the disk killed a sensitive T12 streptococcus. It was said not to be antibody and leucocytes are not necessary for this bactericidal activity as they are for M antibody activity.]

A factor with similar properties to the Tillet factor

was also present in the serum of test animals several days after implantation of the disks in spite of the fact that organisms did not always escape from the disk capsule. Group A antibody was also present.

[The M antibody was not sought and there is no mention of the specificity of the Tillet factor or its relation to this antibody.] *M. Patricia Jevons*

FOLEY, Marie J.; SMITH, Mary R.; WOOD, W. B., Jr. **Studies on the Pathogenicity of Group A Streptococci. I. Its relation to Surface Phagocytosis** [FOLEY, SMITH & WOOD]. *J. Exper. Med.* 1959, Oct. 1, v. 110, No. 4, 603–16, 4 figs. on 4 pls. [37 refs.] **II. The Antiphagocytic Effects of the M Protein and the Capsular Gel** [FOLEY & WOOD]. *Ibid.*, 617–28, 1 fig. on pl. [34 refs.]

I. 4 different strains of group A streptococci were tested for virulence in mice and rats, and for their susceptibility to phagocytosis by mouse or rat leucocytes either on surfaces such as filter paper or glass, or in suspension in rotated tubes. A matt, M-containing and encapsulated strain resisted phagocytosis under all conditions; an M-deficient variant of it was susceptible to phagocytosis on a filter paper surface but not in suspension. Resistance to surface phagocytosis seemed better correlated with virulence, as measured by mortality after intraperitoneal injection, than phagocytosis on glass or in suspension. Examination of the peritoneal exudate of mice after such injection showed much less phagocytosis of the virulent than of the avirulent strains.

II. Cultures of the 4 strains of streptococci were treated with trypsin, to destroy the M antigen, or hyaluronidase, to destroy the capsule, and then tested for their susceptibility to phagocytosis. In a virulent strain, rich in M and capsule, treatment with either enzyme increased susceptibility to phagocytosis on filter paper, but treatment with both was needed to promote phagocytosis in suspension. The trend of these results was confirmed with the less virulent strains, suggesting that both capsule and M antigen conferred resistance to phagocytosis on a streptococcus. In one strain, M antigen, although present, seemed to have relatively little antiphagocytic effect. [In both these papers there is often an easy transition from "correlation" to "relation" and no attempt is made to resolve the differences between the authors' results on the lack of phagocytosis of avirulent strains in suspension and other experience in this field.] *R. E. O. Williams*

PANOS, C. & BARKULIS, S. S. **Streptococcal L Forms. I. Effect of Osmotic Change on Viability.** *J. Bacteriology.* 1959, Aug., v. 78, No. 2, 247–52, 3 figs. [14 refs.]

Growth characteristics are reported of the penicillin-induced L form obtained from a non-typable group A *Streptococcus pyogenes* designated AED [this *Bulletin*, 1955, v. 30, 362]. Upon prolonged subculture in liquid medium the macroscopic growth features resembled those of the parent organism. The effect of various solutions on structural integrity and viability were investigated by centrifugation of the L form, resuspension and washing in the solution under test and finally transfer to the standard growth medium. Sucrose solutions (0·25 to 0·88 M) were osmotically protective but distilled water and glycerol (0·3 M to 1·4 M) failed to support viability or structural integrity of the L. form. *H. Gooder*

SKADHAUGE, K. & PERCH, Beate. **Studies on the Relationship of some Alpha-Haemolytic Streptococci of Human Origin to the Lancefield Group M.** *Acta Path. et Microb. Scandinavica.* 1959, v. 46, No. 3, 239–50. [10 refs.]

10 strains of α-haemolytic streptococci from the mouths of healthy individuals or from the blood of patients with bacterial endocarditis were found to give good precipitation reactions with a Group M serum. These strains were studied in comparison with typical β-haemolytic animal strains of Group M. Several physiological differences were recorded. The details of the serological tests cannot easily be summarized, but a thermostable antigen common to many of the strains was observed, which appeared to differ from the typical Group M antigen, which has been found to be thermolabile. *R. E. O. Williams*

HUGH, R. **Motile Streptococci isolated from the Oropharyngeal Region.** *Canadian J. Microbiol.* Ottawa. 1959, Aug., v. 5, No. 4, 351–4, 8 figs. on pl. [29 refs.]

During a systematic examination of 820 oropharyngeal specimens 5 motile strains of streptococci and 70 strains of non-motile Group D streptococci were isolated.

The motile organisms were α-haemolytic on rabbit blood agar and were culturally and biochemically similar to non-motile Group D streptococci; they also reacted with Group D antiserum. Long chains were produced in broth and flagella could be stained by Leifson's technique. Motility was best seen in logarithmic phase of growth at room temperature. Organisms usually had 1 or 2 flagella originating at the distal end of a pair of cocci and flagella length varied from 1 to 10 times the diameter of the coccus.

The author regards these organisms as variants of *Strep. faecalis.* *M. Patricia Jevons*

AUSTRIAN, R. **The Prevalence of Pneumococcal Types and the Continuing Importance of Pneumococcal Infection.** *Amer. J. Med. Sci.* 1959, Aug., v. 238, No. 2, 133–9. [12 refs.]

From 1952, patients in a Brooklyn, N.Y., hospital with acute disease of the respiratory tract or fever of unknown origin had sputum, or in its absence, nasopharyngeal and oropharyngeal swabs, examined for pneumococci. Material was injected intraperitoneally into mice. All pneumococci were typed. The number of strains isolated each year over the 5 years 1952–7, from the 225-bed service, varied between 155 and 365. The 3 commonest types were, in order, 3, 7, and 1,

and altogether 49% fell into types 1–8 inclusive. Type 2 was rare. On average about 45 cases of pneumococcal bacteriaemia were recognized each year.

R. E. O. Williams

PAKUŁA, R., WALCZAK, W., FLUDER, Z. & ZAKRZEWSKI, K. Warunki środowiskowe transformacji dwoinek zapalenia płuc. [**The Environmental Conditions of Transformation of Pneumococci. The Rôle of Albumin**] *Acta Microbiologica Polonica.* 1958, v. 7, No. 2, 97–104. English summary.

The authors have investigated the effect of preparations of human and bovine serum albumin upon the transformation of pneumococci by deoxyribonucleic acid extracted from a streptomycin-resistant strain. The transformation was carried out in a medium consisting of meat extract, neopeptone, yeast extract and 0·025% glucose which had been adsorbed 3 times with activated charcoal, and to which was added 0·4 cc. of transforming factor solution containing 10 μgm. per cc. DNA, and 0·4 cc. of a 2·5% solution of the albumin solution under investigation. After growth for 6–8 hours the occurrence of transformation was detected by plating on agar containing 250 μgm. of streptomycin per cc. Under these conditions transformation was brought about by 1 of 4 albumin preparations. The inactive preparations did not inhibit the transformation brought about by the active preparation. Electrophoretic analysis showed that the latter contained 8% of beta globulin and agglutinated pneumococci in the R phase. Transformation could also be obtained in the presence of antiserum containing R-agglutinin, which was possibly an essential factor in transformation to streptomycin resistance.

D. J. Bauer

RAPPAPORT, H. P. & GUILD, W. R. **Defined Medium for Growth of Two Transformable Strains of** *Diplococcus pneumoniae. J. Bacteriology.* 1959, Aug., v. 78, No. 2, 203–5.

2 transformable strains of *Streptococcus pneumoniae* were successfully grown in a chemically defined medium consisting of amino acids, vitamins, inorganic salts, glucose and sodium pyruvate. All chemicals used were high purity commercial preparations. A method is presented for the isolation of auxotrophic mutants of these strains.

H. Gooder

RAUNIO, V. **Haemagglutination caused by Pneumococci.** *Ann. Med. Exper. et Biol. Fenniae.* Helsinski. 1959, v. 37, No. 3, 243–50, 2 figs.

Virulent pneumococci of various types were injected into the amnion and allantois of chick embryos 7–12 days old. After 1–4 days at 37°C. fluids were harvested. Growth as judged by turbidity of fluid and colony counts was best in the allantoic sacs of 7–8-day embryos all of which died within 24 hours.

Haemagglutination tests were carried out on the fluids by using chick cells, and titres varied between 1/32 and 1/2048, only cells from 5 of 8 chicks were satisfactory and cells of most other common species were not agglutinated. The titre rose with the temperature at which the test was performed. It was maximum at 56° but the factor was inactivated at this temperature. Shaking the fluid dilutions at room temperature or 37° increased the titre and the haemagglutinating factor could not be eluted from the red cells. After removal of pneumococci from the allantoic fluid by centrifugation the supernatant retained its haemagglutinating activity and was capable of lysing heat-killed pneumococci, suggesting that it was identical with the pneumococcal autolytic enzyme. Haemagglutination was not inhibited by pneumococcal antiserum. Strains of numerous groups of streptococci failed to agglutinate chick red cells.

M. Patricia Jevons

AUSTRIAN, R.; BERNHEIMER, Harriet P.; SMITH, E. E. B.; & MILLS, G. T. **Simultaneous Production of Two Capsular Polysaccharides by Pneumococcus. I. Properties of a Pneumococcus manifesting Binary Capsulation** [AUSTRIAN & BERNHEIMER]. *J. Exper. Med.* 1959, Oct. 1, v. 110, No. 4, 571–84, 6 figs. on 4 pls. [14 refs.] **II. The Genetic and Biochemical Bases of Binary Capsulation** [AUSTRIAN, BERNHEIMER, SMITH & MILLS]. *Ibid.*, 585–602, 1 fig. [30 refs.]

i. "Pneumococci manifesting binary capsulation have been produced through the agency of transformation reactions, Such cells produce two capsular polysaccharides which are closely similar if not identical with those produced by corresponding singly capsulated strains.

"Binary capsulation in pneumococcus provides a new basis for serologic cross-reactivity in this species."

ii. "Study of the capsular genome of pneumococcus has shown that it controls a multiplicity of biochemical reactions essential to the synthesis of capsular polysaccharide.

"Mutation affecting any one of several biochemical reactions concerned with capsular synthesis may result in loss of capsulation without alteration of other biochemical functions similarly concerned.

"Mutations affecting the synthesis of uronic acids are an important cause of loss of capsulation and of virulence by strains of pneumococcus Type I and Type III.

"The capsular genome appears to have a specific location in the total genome of the cell, this locus being occupied by the capsular genome of whatever capsular type is expressed by the cell.

"Transformation of capsulated or of non-capsulated pneumococci to heterologous capsular type results probably from a genetic exchange followed by the development of a new biosynthetic pathway in the transformed cell. The new capsular genome is transferred to the transformed cell as a single particle of DNA.

"Binary capsulation . results from the simultaneous presence within the pneumococcal cell of two capsular genomes, one mutated, the other normal. Interaction between the biochemical pathways controlled by the two capsular genomes leads to augmentation of the phenotypic expression of the product controlled by one

and to partial suppression of the product determined by the other.

"Knowledge of the biochemical basis of binary capsulation can be used to indicate the presence of uronic acid in the capsular polysaccharide of a pneumococcal type the composition of the capsule of which is unknown."

UDAKA, S., KOUKOL, Jane & VENNESLAND, B. **Lactic Oxidase of Pneumococcus.** *J. Bacteriology.* 1959, Nov., v. 78, No. 5, 714–25, 3 figs. [28 refs.]

SKORKOVSKÝ, B. Rhodanidový test k odlišení enterokoků. [**A Thiocyanate Test for identifying Enterococci**] *Československ. Epidemiol., Mikrobiol., Imunol.* Prague. 1959, v. 8, No. 4, 251–4. [10 refs.] English summary (4 lines).

A method of distinguishing enterococci from other species of streptococci has been developed, which is based on the observation that the growth of α- and β-haemolytic streptococci is inhibited by potassium thiocyanate. The strain to be investigated is grown in a medium consisting of 100 cc. *p*H 7·3 meat-peptone broth, 0·75 gm. each of lactose and maltose, 0·1 cc. of a 3% alcoholic solution of bromothymol blue and 2·25 gm. of potassium thiocyanate. If growth occurs the medium turns from blue to yellow, and the result is observed after incubation for 16–24 hours. None of 163 strains of *Streptococcus viridans* grew in the medium, and only 2 of 49 strains of β-haemolytic streptococci, whereas 234 of 236 strains of enterococci grew and produced a change of colour. *D. J. Bauer*

TARSHIS, M. S. **Further Investigation on the Selective Activity of Fluid Thioglycollate Medium for Group Differentiation of *M. tuberculosis*, Anonymous (Atypical) Acid-Fast Bacilli, and Saprophytic Mycobacteria.** *J. Lab. & Clin. Med.* 1959, Oct., v. 54, No. 4, 630–33. [13 refs.]

50 freshly isolated strains of tubercle bacilli, 50 strains of atypical acid-fast bacilli (20 photochromogens and 30 scotochromogens), and 5 saprophytes were selected for this study, each strain being subcultured from a 14-day-old Dubos culture into the fluid thioglycollate medium. None of the tubercle bacilli grew in 11–39 days; all the saprophytes grew in 1 day.

All these strains were catalase-positive, but only the tubercle bacilli were peroxidase- and neutral-red-positive. Except for the 2 strains of *Mycobacterium phlei* and the 1 strain of *Myco. butyricum*, only the tubercle bacilli grew in cord formation.

Growth in the thioglycollate medium is therefore considered to be a valuable test for differentiating these 3 classes of mycobacteria, especially when used in conjunction with these other tests but during prolonged incubation a crystalline deposit develops and this needs distinguishing from the growth of the bacilli.

S. R. M. Bushby

SPITZNAGEL, J. K. & SHARP, D. G. **Magnesium and Sulfate Ions as Determinants in the Growth and Reproduction of *Mycobacterium bovis*.** *J. Bacteriology.* 1959, Oct., v. 78, No. 4, 453–62, 14 figs. [32 refs.]

This study was made on BCG growing in a modified Dubos medium, with magnesium chloride and potassium sulphate as the sources of the magnesium and sulphate ions.

Turbidimetric measurements showed that both ions were necessary for maximum growth; with equi-molar concentrations, 0·4 mM per litre was optimum but within limitations there was an inverse relationship between the 2 ions, and both salts were slightly inhibitory at 4 mM per litre.

In cultures in which neither ion was present, the bacilli were abnormally long, being 6–10μ by the 10th day, and they were very scanty. The bacilli stained irregularly with Ziehl-Neelsen stain but the non-acid-fast areas were stainable with Sudan black; the sudanophilic material was extractable with pyridine. Staining with HCl-Giemsa, HCl-azure A SO_2, and Feulgen stains revealed nucleus-like bodies closely associated with these sudanophilic areas, and electron micrographs showed vacuole-like structures in similar positions and of similar solubility to the sudanophilic material. The cell walls of the bacilli appeared to have developed normally.

Bacilli grown in medium containing both ions stained normally and took the Sudan black very poorly. They contained in the terminal regions, volutin granules and electron-dense granules, which resembled the mitochondria of mammalian cells; these regions also showed marked electron scattering, believed to be due to metaphosphates.

The abnormal lengthening of the bacilli was shown to be associated with sulphate rather than magnesium deficiency. Apparently magnesium deficiency did not interfere with DNA or other nuclear constituents but it prevented the formation of volutin and metaphosphates; sulphate deficiency apparently affected the synthesis of RNA but not of volutin.

These observations are discussed briefly in relation to those of other workers. *S. R. M. Bushby*

GALE, D. **The Control of Contamination in the Cultivation of Tubercle Bacilli. II. The Addition of Chloramphenicol to Egg-Containing Media.** *Antibiotics & Chemotherapy.* New York. 1959, Oct., v. 9, No. 10, 627–30, 2 figs.

Chloramphenicol was added before inspissation to ATS egg medium in concentrations of 50, 25 and 10 μgm. per ml., and specimens of sputum, gastric washings and urine were inoculated after treatment with 4% caustic soda onto this medium alone and onto slopes containing one of these concentrations of the antibiotic.

The results showed that although the chloramphenicol medium was contaminated less often than the plain medium, the growth of tubercle bacilli was often reduced. Nevertheless, the author considers that the medium, containing 25 μgm. chloramphenicol per ml. can be recommended for routine use but it should be

used in conjunction with the plain medium. This recommendation is based on the fact that of the 540 specimens of the series in which growth on the medium containing 25 μgm./ml. was compared, 148 were contaminated but 80% of these were more contaminated on the plain medium than on the antibiotic medium, only 14% showing the reverse, and that of the 54 positive specimens in this series, 70% gave better results on the plain medium as compared with 22% on the antibiotic medium. [For Part I, see this *Bulletin*, 1959, v. 34, 161.] *S. R. M. Bushby*

GÄNGEL, G. Untersuchungen über die Wirkung des Vitamin-B-Komplexes auf das Wachstum von *Mycobacterium tuberculosis* und den Verlauf der Meerschweinchen-Tuberkulose. [**The Effect of the Vitamin B Complex on the Growth of *Mycobacterium tuberculosis* and on Tuberculosis in Guineapigs**] *Arch. f. Hyg. u. Bakt.* 1958, Oct., v. 142, No. 7, 481–93. [20 refs.]

In a preliminary orientating experiment, the author found that a proprietary vitamin B preparation, Polybion, inhibited the growth of a saprophytic acid-fast bacillus but not of strains of *Escherichia coli*, *Staphylococcus aureus* or a haemolytic streptococcus. The preparation contained 2·5 mgm. thiamine, 0·5 mgm. riboflavine, 7·5 mgm. nicotinamide, 1·5 mgm. calcium pantothenate and 1 mgm. pyridoxine per ml. This led to investigation of the effect of the vitamin B complex on cultures of *Mycobacterium tuberculosis* and on experimental tuberculosis in the guineapig.

In the first experiment, 1 ml. volumes of a suspension containing 5 mgm. of tubercle bacilli in 5 ml. saline were added to 5 ml. of a 1:2 dilution of Polybion. After periods of 1–24 hours, the tubes were centrifuged and the deposit cultured on Hohn's egg medium. Growth of the tubercle bacilli was not affected by 4 hours' exposure to the vitamin B preparation, but little growth occurred after 8 hours' exposure and none after 24 hours' exposure. Later experiments confirmed this inhibitory effect, the extent of which varied with the concentration of the vitamins and the duration of exposure. Similar results were obtained when the exposed organisms were transferred into fluid Kirchner's medium.

When various concentrations of Polybion were added to the egg medium, inhibition of the growth of tubercle bacilli was observed up to a concentration of 1 in 25 but normal growth occurred with a 1 in 50 concentration. Experiments with the component vitamins singly and in various combinations suggested that thiamine and pantothenic acid were mainly responsible for the inhibitory effect.

The author next investigated the effect of Polybion on the course of experimental tuberculosis in the guineapig. In an experiment in which 2 control animals died after 20 and 22 days respectively, feeding Polybion in 1 ml. amounts for 6 days before inoculation prolonged life, although when the 2 animals so treated were killed after 6 weeks they showed evidence of generalized tuberculosis. When 1 ml. of Polybion was given daily for 21 days before inoculation with tubercle bacilli. 2 test animals survived for 67 and 79 days respectively. The administration of vitamin B to guineapigs after they had been inoculated with tubercle bacilli had no delaying effect on the outcome of the disease. *B. Moore*

COLETSOS, P. J., with the technical collaboration of E. ORIOT, N. DE REGEL & M. G. MACHAVOINE. Étude des facteurs intrinsèques de *Mycobacterium tuberculosis* expliquant l'état de quiescence et les échecs de culture sur milieux usuels. [**Study of Intrinsic Factors of *Mycobacterium tuberculosis* implied in the Quiescent State and Cultivation Failures on Routine Media**] *Ann. Inst. Pasteur.* 1959, Sept., v. 97, No. 3, 290–306. [20 refs.]

The first part of this paper is a discussion concerning the factors that may affect the growth of tubercle bacilli from tissues, before and after contact with anti-tuberculous drugs. The author suggests that drugs not only induce in the bacillus a quiescent state which may make the growth *in vitro* of the bacillus difficult but also a "sensitive persistent" state, which under certain conditions may accelerate the growth.

Experiments are described in which inocula of about 100 tubercle bacilli were placed on Jensen medium containing PAS in concentrations ranging from 0 to 10 μgm. per ml. and to which PAB was added at various intervals ranging from 2 to 30 days; in one of the experiments monkey liver pulp was also added with the PAB. In the absence of PAB, growth was partially inhibited by 0·05 μgm. PAS per ml. and completely by 0·1 μgm. per ml. but when PAB was added after 2–4 days' incubation, the growth in the presence of 10 μgm. PAS per ml. was equal to that on the medium without PAS and on the medium containing 2 μgm. PAS per ml. it was even greater. This reversal and enhancing effect was much less if the interval between inoculating and the adding of the PAB was extended and it was still apparent after 30 days. However, when the liver was added with the PAB the resuscitation effect was further increased for the bacilli then grew in the presence of 5 μgm. PAS per ml. even when the interval was 21 days and in 2 μgm. per ml. when it was 30 days.

PAS has been reported to have a temporary stimulating effect on tubercle bacilli, as shown by an increase in the oxygen-uptake, and the author suggests that this effect persists after neutralization by the PAB. As for the resuscitating effect of the liver it is interesting to note that of the various substances that activate the apparently dead bacilli present in pathological specimens after prolonged chemotherapy, monkey liver has, in the author's experience, proved especially effective. He suggests that the change induced in this bacilli by chemotherapy is an involution drawing them nearer to Hansen's bacillus. *S. R. M. Bushby*

TAKEYA, K., YOSHIMURA, T., YAMAURA, K. & TODA, T. **Studies on the Biologic Properties of Mycobacteriophage.** *Amer. Rev. Respiratory Dis.* 1959, Oct., v. 80, No. 4, Pt. 1, 543–53, 4 figs. [12 refs.]

The 9 strains of bacteriophage were isolated from soil enriched with mycobacteria and manured with

human or chicken faeces; various species of mycobacteria were used as the host.

The plaques of the individual phages varied in size and that of phage A4 was hazy, the others being clear. 5 of the phages (A2, A3, B1, B2 and B3) were active against virulent tubercle bacilli; of 26 human strains A2, B1, B2 and B3 were active against 25; of 13 bovine strains B1 was active against 12, B2 and B3 against 11 and A2 against 9; the 5th strain, A3, was active against 24 of the human strains but against none of the bovine strains. Neutralization tests with antiserum to B1 showed that B1, B2 and B3 were antigenically very closely related and that A2 and A3 were less closely related to them; the other phages were antigenically unrelated.

Virulent strains of avian tubercle bacilli were also susceptible to these 5 phages but avirulent strains were susceptible to all the phages. Strains of human tubercle bacilli, resistant to isoniazid, PAS or streptomycin were also susceptible to these phages, but attenuated strains were less susceptible.

An unsuccessful attempt was made to detect lysogenic strains among 20 saprophytic strains.

S. R. M. Bushby

TUNÇMAN, S. & PACKALÉN, T. **Specific and Non-Specific Inhibition of Fibro-Blastic Growth in Tissue Cultures of Lung and Spleen Explants from Tuberculin-Sensitized Guineapigs.** *Acta Path. et Microb. Scandinavica.* 1959, v. 46, No. 4, 361–7. [24 refs.]

"The fibro-blastic growth in tissue cultures of lung and spleen explants from tuberculin-sensitized guineapigs was inhibited by specific tuberculin (PPD) as well as non-specific bacterial products (*E. coli* culture filtrate) in concentrations, which did not inhibit the growth of fibro-blasts from normal guineapigs."

TSUMITA, T., MATSUMOTO, R. & MIZUNO, D. **The Nature of the Middlebrook-Dubos Haemagglutination Antigen of** *Mycobacterium tuberculosis.* *Japanese J. Med. Sci. & Biol.* 1959, June, v. 12, No. 3, 167–70, 1 fig.

"The Middlebrook-Dubos haemagglutination antigen could be extracted from *Mycobacterium tuberculosis, Aoyama B*, a virulent human type, and was purified by starch zone electrophoresis and ultracentrifugation.

"It was highly active for the sensitization of red cells in a concentration of 0.5–0.25 μg/cc as well as for the M-D inhibition reaction. The purified antigen was of polysaccharide nature with a macromolecular size. $S^{1}\%$ value of the antigen was 26.1 S."

OLIVER, A. J., STAINTON, J. & TAYLOR, P. J. **A Simple Filter-Paper Disc Method for determining the Sensitivity of** *Myco. tuberculosis.* *J. Clin. Path.* 1959, Sept., v. 12, No. 5, 444–7.

This method consists of impregnating 6 mm. disks with 10 μgm. streptomycin, 5 μgm. PAS, 1 μgm. isoniazid, 1 μgm. Dipasic and 25 μgm. viomycin, and placing 1 of each of them on to the top of Löwenstein-Jensen slopes, inoculated with a standard quantity of bacilli (approximately 0·015 ml. containing 5×10^8 bacilli per ml.). The size of the zone of inhibited growth is read after 10–14 days' incubation.

Reliability of the method was tested by examining 94 strains for sensitivity to streptomycin, 205 strains for sensitivity to streptomycin, PAS and isoniazid, and 49 and 52 strains for sensitivity to Dipasic and viomycin, respectively, using both this diffusion method and a serial dilution method; good agreement was found between the 2 methods. The diffusion method gave reproducible results in the same batch of medium, but the size of the zones varied with the size of the inoculum and slightly, but not significantly, with different batches of the medium.

Zones of 10–50 mm. (measuring from the centre of the disk to the edge of the zone) were given by sensitive strains, 5–9 mm. zones, depending on the drug, by partially resistant strains and zones less than 5 mm. with fully resistant strains. *S. R. M. Bushby*

TIRUNARAYANAN, M. O., VISCHER, W. A. & BRUHIN, H. **Amithiozone: Mechanism of Action and Resistance Development by Mycobacteria.** *Amer. Rev. Respiratory Dis.* 1959, Oct., v. 80, No. 4, Pt. 1, 559–68, 2 figs. [27 refs.]

A mutant of H37Rv resistant to amithiozone [thiacetazone] was observed to produce a diffusable yellow pigment, similar to that of the naturally resistant saprophytes, but the examination of several other resistant strains of human and bovine origin showed that this property was probably unique.

Experiments showed that the production of the pigment was associated with the carbon source in the medium; it was produced in Youmans medium but in Dubos medium only when 1% carbohydrate (glucose, glycerol or *meso*-inositol) was added. Several carbohydrates supported growth in Youmans medium, but the facts that *meso*-inositol was the best for encouraging pigment production, and that the colour was then reddish, suggested a relationship with riboflavin formation. Also, tests with different nitrogenous substances including nitrite, nitrate, ammonium salts, amides and amino-acids showed that histidine, a known precursor in riboflavin synthesis, alone stimulated production of the pigment.

These indications of a flavin-nucleotide being formed were further supported by the greatly stimulated production of pigments by the saprophytes when adenine, adenosine, hypoxanthine or 4-amino-5-imidazole-carboxamide was added. However, these substances had no effect on the pigment production by the tubercle bacillus, and fluorimetric analysis of the filtrates of the pigmented cultures did not indicate the presence of any significant amounts of the vitamin. Experiments with D-amino acid oxidase also failed to demonstrate any co-enzyme in the cultures.

As spectroscopic examination revealed a peak absorption at 265 mμ., the effects of several purine antagonists were investigated; 6-mercaptopurine showed the maximum inhibitory effect against the tubercle bacillus and in the presence of sub-inhibitory concentrations of

2,6-diaminopurine the saprophytes produced greater amounts of pigment in the presence of adenine, hypoxanthine and their derivatives, suggesting that the pigment is a product of a block in the metabolism of the purines.

The pigment does not correspond to any others known to be associated with tubercle bacilli and the authors suggest it is a polymerization product of amino-imidazole-ribotide together with a related purine derivative. They suggest that the thiosemicarbazones enter purine metabolism of susceptible tubercle bacilli which utilize methionine in nucleic acid synthesis, giving rise to fractions incapable of reproduction. Loss of a mechanism involving the biosynthesis of hypoxanthine using methionine would then produce amithiozone-resistant variants, and the location of the genetic blocks could be at different places with different strains, leading to the evolution of pigmented or non-pigmented resistant variants. *S. R. M. Bushby*

Debré, R., Noufflard, H. & Foussereau, S., with the technical collaboration of M. L. Guivarc'h. "Chimioprophylaxie" par l'isoniazide et immunité antituberculeuse. Contribution à l'étude expérimentale. [**Chemoprophylaxis with Isoniazid and Antituberculous Immunity. An Experimental Contribution**] *Rev. Française Études Clin. et Biol.* Paris. 1959, v. 4, No. 1, 32–9, 14 figs. [11 refs.]

In this study guineapigs were injected intradermally in the left side with 0·1 ml. of 10^{-4} dilution of a Dubos culture of H37Rv, and 35 days later a similar injection was made into the right side. The immunity developing from the first injection was measured by the extent of suppression of the second injection, this being assessed by the amount of the ulceration and the degree of involvement of the draining lymph gland. The results show that a high degree of immunity was conferred but if the guineapigs were treated with 10 mgm. isoniazid per kgm. each day from the day of infection for 27 days, the immunity was almost completely suppressed, and that there was partial suppression when this treatment was delayed for 10 days and continued for only 17 days. *S. R. M. Bushby*

Cerbón, S., J., Bojalil, L. F. & Pérez-Tamayo, R. **Reinfection in Guinea Pigs with Tuberculosis arrested by Chemotherapy.** *Amer. Rev. Respiratory Dis.* 1959, Oct., v. 80, No. 4, Pt. 1, 554–8. [15 refs.]

In these experiments guineapigs were infected with 0·2 mgm. H37Rv and some of the animals were treated with isoniazid and streptomycin. 60 days after infection the animals were reinfected with 0·02 mgm. of a variant of H37Rv, resistant to 100 μgm. viomycin per ml., thus enabling the bacilli of the primary infection to be readily distinguished from those of the secondary infection. The reinfection was made once, twice or thrice with 5-day intervals between doses.

In untreated animals in which the reinfection was given once the reinfecting bacilli remained at the site of the infection but in treated animals these bacilli were recovered, 10–30 days after reinfection, from the draining lymph glands and the spleen but not from the liver or lungs. When the reinfection was given more than once, dissemination of the reinfecting bacilli occurred, the bacilli being recovered from all of these organs, and this dissemination occurred irrespective of whether there had been treatment or not.

In the treated and non-reinfected animals the tissues showed little histological evidence of infection, but in the animals reinfected only once there was definite evidence of disease in the draining lymph gland, and in those reinfected twice there was evidence of disease in all the tissues.

The tuberculin state of the animals did not correlate with their immunity, demonstrated by localization of the second infection; in fact, 5 of the animals showing little or no spread of the resistant bacilli were tuberculin-negative. *S. R. M. Bushby*

Toyohara, M., Kudoh, S. & Obayashi, Y. **Studies on the Effect of Isoniazid upon the Antituberculous Immunity induced by BCG Vaccination.** *Tubercle.* 1959, June, v. 40, No. 3, 184–91, 4 figs.

There is a conflict of opinion about the means by which BCG vaccine produces immunity against tuberculosis. Some workers consider that the level of immunity depends on the multiplication of the bacilli in the tissues of the host and others that this is not an essential factor [this *Bulletin*, 1955, v. 30, 583]. In order to answer some of these questions the authors carried out experiments on the effect of the administration of isoniazid on the immunity induced by BCG in guineapigs.

The vaccines used were made from either dried living or heat-killed BCG; 1 mgm. of the reconstituted living vaccine contained 8×10^7 viable units, the dose being 0·5 ml. of a 10 mgm./ml. concentration given subcutaneously. The same dose of heat-killed vaccine was used. The challenge infection was a culture of a human type tubercle bacillus which had been dried by freezing. After reconstitution a dose of 0·5 ml. was given subcutaneously (0·01 mgm. of a concentration of 15×10^6/mgm. viable units). Isoniazid was given subcutaneously each day for various stated periods in dosage of 0·5 ml. of a 10 mgm./ml. solution in water. The degree of tuberculin sensitivity induced in each animal was determined every 2 weeks after vaccination (100 TU of OT). The test was repeated 2 and 4 weeks after challenge infection.

There were 6 groups of guineapigs, each of 9–12 animals (total 61). In the animals of the group in which the administration of isoniazid was started 5 days before vaccination the degree of immunity was reduced to such an extent (as judged by macroscopic and microscopic findings at autopsy and by quantitative culture of tissues) that it differed little from that in the group vaccinated with killed BCG.

There was evidence that isoniazid decreased the degree of tuberculin sensitivity present whether it was given before or 2 weeks after vaccination. This finding supports the view that tuberculin sensitivity and immunity are not always parallel.

It was found that when isoniazid was first given 2 weeks after vaccination the development of immunity seemed little affected although the degree of tuberculin sensitivity was clearly reduced. The degree of immunity of the group infected 2 weeks after vaccination, was obviously lower than that of the group infected 4 weeks after vaccination, during which period isoniazid was administered.

An additional finding was that there was less extensive tuberculous disease in the group vaccinated with heat-killed BCG than in the unvaccinated control group.

H. G. Calwell

BLOCH, H. & FERREIRA, I. **The Effect of Adjuvants on Antituberculosis Vaccination with Live Mycobacteria.** *J. Immunology.* 1959, Oct., v. 83, No. 4, 372–7, 2 figs. [15 refs.]

The authors have previously shown that living vaccines of attenuated tubercle bacilli give better protection than killed vaccines [this *Bulletin*, 1955, v. 30, 583], and they now report experiments in which living bacilli have given less protection when suspended in an adjuvant than when suspended in saline.

In the first experiment an injection of BCG suspended in a mixture of 85% Bayol F and 15% Arlacel afforded no protection to mice against a challenge dose of the Ravenel strain of bovine tubercle bacilli, given 3 weeks later, whereas a similar dose of the BCG suspended in saline gave very definite prolongation of life. In the second experiment in which graded doses of H37Ra, ranging from 1·0 mgm. to 0·0001 mgm., were used as the vaccine, at least 10,000 times more bacilli were required to give similar protection when the vaccine was suspended in the adjuvant than when it was given in saline.

The results of further experiments showed: (1) that large doses of BCG, *i.e.* 0·1 ml. of a 10-times concentrated Dubos culture, afforded somewhat less protection than a dose 1,000-times smaller; and (2) that 2 doses at an interval of 1 week were less effective than a single dose, except when the second dose was extremely small, *i.e.* 0·1 ml. of a 10^{-3} dilution of a Dubos culture.

In previous experiments the authors had noted that mice showed a fall in weight immediately after challenge, an observation which they consider might be an allergic phenomenon. In the present experiments no loss of weight occurred after vaccination, and the falls that occurred after challenge were greater in the groups of mice that were least protected than in those that were more highly protected.

In the discussion of these results the authors consider 4 possible explanations to account for the decreased activity of the living bacilli when suspended in the adjuvants. These are: (1) that the adjuvant removes a cellular constituent; (2) that the oily vaccines become less disseminated in the mice; (3) that the adjuvants cause uneven stimulation of the various antigens of the bacilli, some of which might have an adverse effect on immunity; and (4) that with the adjuvant the antigenic response is delayed and that the difference between the vaccines would have been less conspicuous if the interval between vaccination and challenge had been longer.

S. R. M. Bushby

McMILLEN, S. & KUSHNER, D. S. **Atypical Acid-Fast Bacilli. III. An Expanded Scheme.** *Amer. Rev. Respiratory Dis.* 1959, Sept., v. 80, No. 3, 434–7. [Refs. in footnotes.]

This scheme is an expansion of the one previously reported by the authors [this *Bulletin*, 1957, v. 32, 1193], and includes 17 species of mycobacteria that might appear in routine specimens. [For 12 of the species only 1 strain of each was examined.]

The species are divided into 2 main groups; Group 1 consists of 8 species of mycobacteria and 2 species of nocardia, all of which were able to grow at 20°C. on the surface of a beef and yeast extract broth; Group 2 consists of 9 species of mycobacteria, none of which were able to grow in this medium at 20°C., although all except *Mycobacterium tuberculosis* and *Myco. paratuberculosis* grew at the bottom of the medium at 37°C. Each of the groups was further divided into 3 subgroups on the basis of pigment production in the presence and absence of light.

	Group 1	*Group 2*
Sub-group A	*Myco. fortuitum* *Myco. smegmatis*	*Myco. tuberculosis* *Myco. paratuberculosis* *Myco. avium* *Myco. bovis* *Myco. intracellularis*
Sub-group B	*Myco. balnei* *Myco. marinum* *Myco. platypoecilus*	*Myco. kansasii*
Sub-group C	*Myco. phlei* *Myco. thamnopheos* *Myco. rhodochrous* *N. asteroides* *N. brasiliensis*	*Myco. marianum* *Myco. scrofulaceum* *Myco. paraffinicum*

The strains were also examined for: (1) degrees of acid-fastness; (2) for the rate and type of growths on Löwenstein-Jensen medium and on nutrient agar containing 7% glycerol; and (3) growth on blood agar (blood), eosin-methylene blue agar (EMB), Cornmeal agar (CM), Littman's oxgall agar (Litt) and Koser's salt agar containing one of the following organic acids: citric, benzoic, succinic, acetic, propionic, pyruvic, lactic, oxalic, tartaric and malic.

All of the strains of Group 1 and only those of the sub-groups B and C of Group 2 grew on blood (except for 3 in subgroup A), EMB and CM media; only 4 species, all belonging to Group 1, grew on Litt medium. All the species of Group 1 and only *Myco. scrofulaceum* of Group 2 were capable of utilizing the first 5 of the organic acids listed above, and all the species except *Myco. tuberculosis* and *Myco. paratuberculosis* utilized the acetate.

The scheme is claimed to permit grouping of unidentified acid-fast bacteria as a preliminary step towards specific identification.

S. R. M. Bushby

WOLINSKY, E. **Chemotherapy and Pathology of Experimental Photochromogenic Mycobacterial Infections.** *Amer. Rev. Respiratory Dis.* 1959, Oct., v. 80, No. 4, Pt. 1, 522–34, 5 figs. [17 refs.]

The experiments described in this paper were designed to determine the effect of 6 antituberculous drugs,

singly or in combination on a standardized mouse infection with the photochromogen isolated in 1957 from patient M. B. at the Trudeau Sanatorium; this strain has been described in detail [see this *Bulletin*, 1957, v. 32, 701]. Groups of mice were infected intravenously with the organism after mouse-passage, and treatment was begun either on the 3rd day and continued for 87 days, or on the 13th day and continued for 39 days; the untreated mice died 13–69 days after infection.

The lesions were widespread and consisted of abscesses in the kidneys and brain, focal and diffuse cellular infiltration in the lungs, and granulomatous lesions in the bones.

Given singly, isoniazid and streptomycin proved the most effective drugs, amithiozone [thiacetazone] and DDS being less beneficial; cycloserine and sulphadiazine were only very slightly effective and streptovaricin was apparently ineffective. In combination with isoniazid, cycloserine, DDS, amithiozone and sulphadiazine were not additive or synergic; streptomycin and isoniazid together were perhaps slightly more effective than separately. PAS was not evaluated.

The main effects of chemotherapy were on the lesions in the kidneys; it had little effect on the viable bacilli in the spleen and liver. *S. R. M. Bushby*

Oehring, H. Toxicitätsbestimmungen bei Diphtherie-Bakterien. **[Determination of Toxin Production by Diphtheria Bacilli]** *Ztschr. f. Hyg. u. Infektionskr.* 1959, v. 145, No. 6, 587–610, 3 figs. [67 refs.]

A considerable collection of strains of *Corynebacterium diphtheriae*, 314 in all, have been tested for toxicity, both by Römer's skin test in albino guineapigs and by Ouchterlony's S method in which strains are tested on plates of 40% serum agar containing graded concentrations of specific antitoxin and halo production recorded. These results are summarized in the 8th table on p. 603.

In the group of strains from clinical diphtheria there were 54 *gravis*, 9 *mitis* and 3 *intermedius* strains. Of these 14 were negative by the Römer test and 8 by the Ouchterlony S test.

Among 194 strains obtained from carriers or from cases diagnosed as ozoena, sinusitis or pharyngitis, there were 20 *gravis*, 169 *mitis* and 5 *intermedius*. Amongst this group 87 were negative by the Römer test and 83 by the Ouchterlony test. The discrepancies between the two tests were considerable, however.

It is suggested that the tests which were positive only in the Ouchterlony plates with the highest concentration of antiserum, *i.e.* 100 units, may have been due to antibody to antigenic products other than toxin. In consideration, however, of the evidence in the literature for the production of toxic elements other than the specific neurotoxin by diphtheria bacilli such as O'Meara's "B" substance, Niggemeyer's hyaluronidase and Eisler's necrotizing factor distinct from lethal toxin, it is claimed that a negative Römer test with positive gel diffusion test may be explained by unduly rapid diffusion of toxin from the site of injection on account of hyaluronidase production, whereas a positive Römer test with negative diffusion test would occur when a strain produced more necrotizing factor or "B" substance than specific neurotoxin.

This leads the author to the general conclusion that a strain positive by either test should be regarded as a toxic strain.

He also describes one undoubtedly saccharose-fermenting strain in confirmation of Frobisher's experience.

[Frobisher's strains were confirmed as undoubtedly virulent and saccharose-fermenting by Johnstone and McLeod [this *Bulletin*, 1950, v. 25, 185] but rejected by Hackenthal [*Ibid.*, 1958, v. 33, 385] as mixed cultures containing *C. xerosis*.] *J. W. McLeod*

Crawford, J. G. & Fishel, C. W. **Growth of *Bordetella pertussis* in Tissue Culture.** *J. Bacteriology.* 1959, Apr., v. 77, No. 4, 465–74, 4 figs. 18 refs.]

Multiplication of *Bordetella* [*Haemophilus*] *pertussis* was found to occur in tissue culture preparations of monkey kidney, HeLa, KB, and a line of human non-malignant nasal epithelial cells designated DMB. The bacteria failed to grow in the nutrient medium 199 alone, in medium 199 containing monkey kidney extract, in the presence of killed supporting cells, or when separated from these cells by a semi-permeable membrane. Cultures infected with *B. pertussis* and incubated up to 12 days in the presence of antibiotics were only overgrown and destroyed by the bacteria 7 to 9 days after removal of the antibiotics. *F. T. Perkins*

Kind, L. S. & Roesner, Luise. **Enhanced Susceptibility of Pertussis Inoculated Mice to Pollen Extract.** *Proc. Soc. Exper. Biol. & Med.* 1959, Apr., v. 100, No. 4, 808–10.

Mice inoculated with pertussis vaccine have an enhanced susceptibility to lethal effects of water soluble extract of rye grass pollen. A mixture of this pollen extract with specific immune rabbit serum or normal rabbit serum is more lethal than the pollen extract alone to inoculated mice. *F. T. Perkins*

Thijsse, G. J. E. & Zwilling-De Vries, J. T. **The Oxidation of Straight and Branched Alkanes by *Pseudomonas* Strains.** *Antonie van Leeuwenhoek: J. Microbiol. & Serol.* 1959, v. 25, No. 3, 332–6. [17 refs.]

i. Kauffmann, F. **On the Principles of Classification and Nomenclature of Enterobacteriaceae.** *Internat. Bull. Bact. Nomencl. & Taxon.* 1959, Jan. 15, v. 9, No. 1, 1–6.

ii. ——. **Definition of Genera and Species of Enterobacteriaceae. Request for an Opinion.** *Ibid.*, 7–8.

(i) Kauffmann argues here that when, in 1936, the Salmonella Subcommittee chose *Salmonella cholerae suis* as type species, all other serotypes were also

regarded as species. He quotes P. Bruce WHITE [*Trop. Dis. Bull.*, 1925, Supplement No. 2, 124] as saying that "They are elementary species *par. excellence*". Although in later reports the Enterobacteriaceae [successor to the Salmonella] Subcommittee dropped the words *genus* and *species*, Kauffmann thinks that these taxonomic terms should now be restored. He regards tribes and genera as biochemically defined divisions within the family, while species are the serotypes, themselves subdivisible into fermentation-types and phage-types.

If this is true for the Salmonella group Kauffmann believes the same principles should be applied to Escherichia, Shigella, Klebsiella, Cloaca, Arizona, Citrobacter, Serratia, Providence, all the serotypes of which would become species. In the Proteus group he would limit the genus *Proteus* to those serotypes defined by Kauffmann and Perch, and would make *Proteus morganii* and *Proteus rettgeri* into genera, *Morganella* and *Rettgerella*.

[The reference to SAVAGE and Bruce WHITE is wrongly cited: the year 1945 is given, instead of 1925.]

(ii) "The Judicial Commission is requested to give an Opinion on the following proposals:

"1. The *Species* of *Enterobacteriaceae* are the established serotypes.

"2. The *genera* are groups of related *species*."

S. T. Cowan

KAUFFMANN, F. **Supplement to the Simplified Kauffmann-White Scheme (I).** *Acta Path. et Microb. Scandinavica.* 1959, v. 45, No. 4, 406–10.

The increasing complexity of the Kauffmann-White scheme for Salmonella serotypes necessitated the production of a simplified scheme that can be used in laboratories that are not international reference centres. The present paper supplements the 1957 revision of the simplified scheme [this *Bulletin*, 1958, v. 33, 162], and lists the sera needed to identify most of the salmonellas that are likely to be encountered. There are 27 "O" sera, 1 Vi and 24 "H" (phase 1) and 2 "H" (phase 2). Small laboratories can omit 2 of the "O" and 9 of the "H" antisera as the serotypes having the corresponding antigens are rare.

From the table of simplified antigenic structures (reproduced here) Kauffmann points out that the combinations of O and H antigens can be seen since every O antigenic complex can be combined with every H antigenic complex of phase 1 together with the "1" complex of phase 2, the e,n complex of phase 2, the L complex, or with each H antigen of the "other antigens" of phase 2. But as soon as new O or H antigens are discovered the number of possible combinations will increase accordingly.

[It is well known that repetition may bring conviction or at least persuade that what is said is correct. These papers are two of Kauffmann's in which he designates as species (emphasized by being printed in italic) Salmonella strains of differing antigenic structure that are generally regarded by workers in this field as serotypes. Kauffmann put forward his new conception of species in the paper reviewed above which has sparked off what is likely to be a lively, even if unprofitable, international discussion (see also TAYLOR, below).] *S. T. Cowan*

Somatic Antigens	Flagellar Antigens	
	Phase 1	Phase 2
A	a	
B	b	1 complex or
C	c	
D	d	
E	h	
11	G	e,n complex or
13	i	
16	k	
17	L	
18	r	L complex or
21	y	
28	z	
30	z_4	other antigens:
35	z_{10}	z_{27}
38	z_{29}	z_{35}
39	z_{35}	z_{37}
40	z_{36}	z_{39}
41	z_{38}	z_{42}
42	z_{41}	z_{43}
43	z_{44}	z_{45}
44	etc.	
45		
47		
48		
50		etc.
51		
52		
etc.		

TAYLOR, Joan. **Why Christen a *Salmonella*?** *Internat. Bull. Bact. Nomencl. & Taxon.* 1959, Oct. 15, v. 9, No. 4, 159–64. [18 refs.]

The various groups in the family Enterobacteriaceae are subdivided on the basis of a partial antigenic analysis. The flagellar, somatic and other surface antigens are defined but the mucoid and fimbrial antigens are ignored. Some of these antigens, *e.g.* Salmonella O antigen 35 and Escherichia O antigen 111, are identical although they occur in different groups. On the other hand the mucoid (M) antigen, the same for all serotypes of the Salmonella group, differs from the M antigen of other groups such as Escherichia. Again the main fimbrial antigen of Salmonella serotypes is the same for the whole group. Extracts of the Enterobacteria may be toxic to animals, and the different members tested seem to produce toxic extracts with the same pharmacological action.

Hybridization may occur between members of different groups (Escherichia × Shigella), and changes in antigenic make-up can be produced by growth in phage or in antisera active against antigens of the test organism.

There is thus considerable evidence that the antigenic make-up of enteric bacteria is relatively easily changed, and it would be illogical to regard Salmonella

serotypes and other interfertile forms as different species.

[This paper concentrates mainly on the question of the unity of the Salmonella group, but it also shows that KAUFFMANN's suggestion [see above] that the serotypes of all enteric bacteria should be regarded as species is incompatible with modern knowledge of antigenic variation.] *S. T. Cowan*

HAENEL, H. & MÜLLER-BEUTHOW, W. Zur Konstanz der fäkalen Flora bei Aufbewahrung. [**The Constancy of Faecal Flora on Storage**] *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1959, Mar.–Apr., v. 5, No. 2, 114–6, 1 fig.

12 stools of normal adults were divided into 3 portions, which were kept respectively at 4°C. for 43 days, 15°C. for 31 days and 24° or 25°C. (both are cited) for 29 days. The specimens were examined bacteriologically on collection and thereafter at intervals of not more than 2 days, with selective media appropriate to the different bacterial groups.

At 4°C. little change in viable counts of the different organisms occurred in the first 6 days of storage. From then until the 20th day, there was a rapid fall in the numbers of anaerobic lactobacilli, staphylococci and fungi. But organisms of the *coli* group, streptococci, microaerophilic lactobacilli and *Proteus* were demonstrable up to the end of the period of observation. Apart from the last-named, the numbers of these organisms showed a steady rise, particularly the streptococci. Paracolon bacilli also became more evident in the later stages of storage, even where none were demonstrated earlier. The numbers of proteolytic organisms remained fairly constant throughout. The *p*H of the specimens remained unchanged up to the 6th day or so and then became more alkaline, to a level of 8·1–8·4.

At 24°C. the changes in flora were similar but more rapid, but in many samples no change in viable counts occurred over the first 3 days. Multiplication of *E. coli* was more marked. At 15°C. an intermediate picture was seen. At all temperatures, specimens of low initial *p*H, 4·2–4·9 showed a steady fall in the numbers of all bacterial groups on storage.

B. Moore

SHIPOLINI, R., KONSTANTINOW, G., TRIFONOWA, A. & ATANASSOWA, S. Apocholat-Citrat-Agar zur Isolierung von *Shigella-* und *Salmonella*-Bakterien. [**Apocholate-Citrate-Agar in the Isolation of Shigellae and Salmonellae**] *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 174, Nos. 1/2, 75–80. [12 refs.]

A medium very similar in composition to SS-agar but containing instead of bile salts 0·85% apocholic acid—a substance chemically related to deoxycholic acid—was tested in parallel with Leifson's medium and water-blue phenol-red agar. Standardized suspensions of a wide range of stock cultures were plated on the 3 media; these included 21 *E. coli* strains, 36 *Shigella* cultures of various types, 21 salmonella strains and a number of *Proteus, Klebsiella,* paracolon and staphylococcal strains. Apocholate-citrate-agar was as effective in suppressing the growth of *E. coli* as Leifson's medium and was less inhibitory to certain pathogens. In particular, some strains of *Sh. dysenteriae* 1 (*shigae*), *Sh. dysenteriae* 2 (*schmitzii*) and of certain salmonellae, notably Salm. Kottbus, Oranienburg, Dublin and occasional typhoid strains, grew on apocholate-citrate-agar, but not on deoxycholate-citrate agar.

The 3 media were then compared in routine practice on a series of 400 stools, including 121 from dysentery convalescents, 203 from normal subjects and 74 from patients. The apocholate-citrate medium yielded 6% of shigella-positive results from healthy subjects, 13·5% from contacts and 80% from patients. The corresponding percentages with Leifson's medium were 4, 9 and 55. *B. Moore*

JOÓ, I., ZALAY, L., KISS, I., FÜVESSY, I. & PUSZTAI, S. Die Massenzüchtung pathogener Darmbakterien. I. Mitteilung: Der Züchtungsvorgang. [**Mass Cultivation of Pathogenic Enteric Bacteria. I. The Technique of Cultivation**] *Zent. f. Bakt. I.* Abt. Orig. 1959 v. 176, Nos. 7/8, 496–513, 2 figs. [59 refs.]

KUSIAK, B. & TUROWSKI, G. Hodowla głębinowa napowietrzna pałeczek *Salmonella typhi* i *Salmonella paratyphi A* i *B*. [**Submerged Aerated Cultivation of *Salmonella typhi* and *Salmonella paratyphi A* and *B***] *Med. Dośw. i Mikrob.* Warsaw. 1959, v. 11, No. 3, 237–47, 5 figs. [15 refs.]

The English summary appended to the paper is as follows:—

"The authors present the manner of conducting submerged aerated cultivation of *Salmonella typhi* and *Salmonella paratyphi* A and B bacilli. For submerged culturing of *S. typhi* and *S. paratyphi* A bacilli the authors employed enzymatic casein hydrolysate enriched by mineral salts containing 200 mg% N-amine. For culturing of *S. paratyphi* B acid hydrolysate of chicken eggs was employed of the same N-amine concentration. The results of the investigations show that in order to obtain the highest possible density of the suspension about 30–40 mld/ml the following parameters should be used: atmospheric air in the amount of 5 litre/min./1 litre of medium, castor oil in the amount of 0.1% as an antifoaming factor, pH between 7.0–7.6, and energetic factor in the form of glucose in the amount of 2–4 g/hour/litre of medium. It proved also necessary to adapt the strains to the medium."

SCHILLER, Barbara, TUROWSKI, G. & KUSIAK, B. Morfologia pałeczek *Salmonella typhi* i *paratyphi A* i *B* w hodowli głębinowej, napowietrzanej. [**Morphology of *S. typhi* and *paratyphi A* and *B* Bacilli in the Process of Aerated Submerged Culturing**] *Med. Dośw. i Mikrob.* Warsaw. 1959, v. 11, No. 3, 249–53, 1 fig. [16 refs.]

The English summary appended to the paper is as follows:—

"Factors responsible for the appearance of changes in the morphological shape of *S. typhi* and *paratyphi*

A and B bacilli are the conditions which take place during the process of aerated submerged culturing. When growing, the bacilli change from small into big, long or thick forms (sometimes oviform) and these in turn into short, rod or spherical-shaped forms. No transition from short and spherical forms into big ones was observed. The bacilli exhibit a tendency for gram-positive staining."

WINKLE, S. & ADAM, W. Über die Lebensdauer von Enterobacteriaceen, insbesondere Salmonellen in künstlich infizierter Margarine. [**Viability of Enterobacteriaceae, especially** *Salmonella* **Strains in Artificially Contaminated Margarine**] *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 174, Nos. 5/6, 364–83, 7 figs. [10 refs.]

In the spring of 1957, several employees of a Bavarian margarine factory went down with typhoid fever after a staff excursion. All the potentially infected margarine was condemned by the public health authorities, but little seemed to be known about the survival of enteric organisms in margarine, hence the present study.

Margarine is an emulsion of a watery and a fatty phase. The watery phase contains skim milk soured after pasteurization, brine, iced water and egg yolk solution. The fatty phase contains various refined vegetable and animal fats and oils. The two phases are emulsified at temperatures of 20°–50°C. for 15–20 minutes. The emulsion then runs over a rotating drum cooled to − 16°C., is scraped off this drum and finally pressed and packaged.

The first experiments were done with the various components of the watery phase. Soured skim milk was inoculated in different experiments with large numbers of *Salmonella typhi, Salm. paratyphi B, Salm. pullorum* or *Escherichia coli*, to give initial viable counts of 10^{10}–10^{11} per ml. *E. coli* could be recovered for 4 days after storage at 18°C., but the salmonellae for no longer than 3 days. In the second experiment, *Salm. typhi* and *Salm. paratyphi B* respectively were inoculated into 50 ml. volumes of a mixture containing 1 part of egg yolk and 2 of water, preserved with 0·75–1% sodium benzoate and 6–8% sodium chloride. By the 9th day both organisms had died out. In the third experiment, the watery phase of margarine was made up at *p*H values 4·5 and 5·5, with and without preservative. Enteric organisms and *E. coli* inoculated into the various preparations survived at most 5 days.

A preliminary experiment on typhoid bacilli and *E. coli* in the fatty phase of margarine showed survival times of 5 and 10 days respectively. Multiplication of an organism in margarine would depend mainly on its lipolytic capacity. A large collection of salmonellae, shigellae, *Arizona*, *Citrobacter* and *Escherichia* strains all failed to break down beef suet, palm oil, cotton-seed oil and whale oil.

In the routine microbiological control of margarine production it is known that bacterial spoilage is greatly influenced by the adequacy of emulsification, and that the smaller the droplet size of the watery phase the more rapidly will bacteria be killed in the final product. This was confirmed in experimental inoculations of margarine with *Salm. pullorum* and *E. coli* followed by homogenization for varying periods. In experiments with enteric organisms the authors found that, in margarine that was adequately homogenized by commercial standards, *Salm. typhi* died out after 5 days in the presence of 0·15% benzoic acid and after 9 days in margarine without preservative. Paratyphoid bacilli died out after 9 days whether or not benzoic acid was present.

In a final series of experiments the authors showed that surface contamination of margarine with pathogens was very unlikely to lead to any risk of enteric infection, apart from the fact that packaging is largely mechanized. *B. Moore*

SCHMIDT, B., LENK, V. & HERZ, F. Versuche zur Gewinnung von Vi-positiven Anteilen aus Vi-negativ erscheinenden Kulturen von *Salm. typhi*. [**Attempts to Isolate Vi-Positive Strains from apparently Vi-Negative** *Salm. typhi* **Cultures**] *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 174, Nos. 1/2, 89–93. [14 refs.]

Attempts were made to isolate Vi-positive forms from Vi-negative typhoid strains, so designated when 20 single colony picks failed to show serological evidence of Vi antigen and the strains were not lysed by the standard typing phages nor by the (I + IV) phage pool. The methods used included mouse inoculation, serial culture in broth containing typhoid antiserum with no Vi antibody and the use of nucleoprotein extracts of Vi-positive strains.

Vi-negative strains were grown for 24 hours in broth and 0·5 ml. volumes of a 1:20 dilution inoculated intraperitoneally into mice. The animals were killed after 60 hours and cultures made from heart blood and peritoneal exudate. Of 16 strains inoculated in this way, 12 were recovered from peritoneal washings or heart blood. From 3 of the 12, Vi-positive colonies were picked and phage-typed, 2 as type C_1 and 1 as type E_1. Further mouse passage of the remaining 9 strains failed to demonstrate the emergence of Vi-positive forms.

Further attempts to detect Vi antigen forming organisms were made by serial culture of typhoid strains in broth containing 4% of Vi-free typhoid antiserum. 12 Vi-negative strains were passed each through 27 passages in this medium. With 1 strain, the 13th passage yielded a Vi-positive strain falling into type D_2; all the other strains remained Vi-negative. In a second experiment of this type, 9 Vi-negative strains were passed through 21 serial passages but no Vi-positive strains were found.

Attempts to pick Vi-positive single colonies from typhoid cultures met with varying success. With some strains, Vi-positive and Vi-negative colonies were present in approximately equal numbers. With other strains, Vi-positive elements were found only after testing 40–50 colonies if at all.

Finally, unsuccessful attempts were made to induce Vi antigen production by treating 20 Vi-negative strains with a nucleoprotein extract of a type A strain of typhoid bacillus. *B. Moore*

SCHMIDT, B. & LENK, V. Zur Epidemiologie und Lysotypie von *Salm. typhi* bei Dauerausscheidern. [**Epidemiological Aspects of Phage Typing of** *Salm. typhi* **Strains isolated from Carriers**] *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 174, Nos. 1/2, 81–8, 2 figs. [17 refs.]

The authors review the results of phage typing of typhoid strains isolated during the years 1954–57 from typhoid carriers infected originally in the course of the pandemic of typhoid fever that raged in Germany in 1945–47. The pandemic affected Mecklenburg in particular. The strains of *Salmonella typhi* from these carriers fell into a wide range of Vi-phage types, including A, C_1, D_1, D_2, D_4, E_1a, E_1b and F_1 apart from untypable strains. The same phage types are still prevalent in Germany. The authors discuss the implications of these findings, notably the point that the heavy incidence of typhoid fever during 1945–47 was due to a combination of factors and not to wide dissemination of one epidemic strain. *B. Moore*

ENGLESBERG, E. & BARON, L. S. **Mutation to L-Rhamnose Resistance and Transduction to L-Rhamnose Utilization in** *Salmonella typhosa*. *J. Bacteriology*. 1959, Nov., v. 78, No. 5, 675–86, 5 figs. [16 refs.]

GLÁZ, E. T. Wirkung der Antibiotika auf *Salmonella typhi* in der menschlichen Galle. [**The Action of Antibiotics on** *Salm. typhi* **in Human Bile**] *Acta Microbiologica*. Budapest. 1959, v. 6, No. 2, 103–11. [12 refs.]

The author first presents in tabular form results from the literature obtained by the antibiotic treatment of persistent typhoid and paratyphoid carriers: the least unsatisfactory results were given by penicillin and tetracycline, each eliminating the condition in about 25% of cases. His own observations consisted of inoculating animal or human bile obtained in various ways and sterilized by heat, to which antibiotics were added in different concentrations, with either a small or a large inoculum of *Salmonella typhi*, and cultivating after incubation to determine in what concentration the organism was killed. Parallel tests were done in broth. Penicillin was the most effective antibiotic, usually sterilizing the small inoculum in a concentration of about 3 units/ml. (in broth 6 units/ml.). Results with streptomycin, chloramphenicol and tetracycline were much more variable in different specimens of bile, and generally better in broth than in bile, but the concentrations required for bactericidal action were in general considerably higher. It is concluded that penicillin is the antibiotic of choice for this purpose. *L. P. Garrod*

COLOBERT, L. & SERVANT, P. L'effet du lysozyme sur l'antigène somatique d'*Eberthella typhi*. [**The Effect of Lysozyme on** *Eberthella typhi* **Somatic Antigen**] *Ann. Inst. Pasteur*. 1959, Oct., v. 97, No. 4, 549–59, 2 figs. [15 refs.]

When lysozyme is allowed to act on suspensions of typhoid bacilli in the presence of ethylene diamine tetra-acetic acid (EDTA) or even heated suspensions without EDTA, a change occurs in the properties of the somatic antigens. In the case of heated bacteria treated with lysozyme it would seem that the bacterial bodies that persist (coagulated protoplasts) have lost their agglutinability; they fail to show any agglutination in the presence of a homologous O antiserum. With Boivin types of lipopolysaccharide extracts, whether treated by heat or with EDTA, lysozyme reduces the reacting power of the antigen, but the combining power would seem to be more affected by lysozyme when the antigens are treated with EDTA. Classical typhoid strains, O 901 and Ty 2, were employed in these experiments and the results indicate that the effect of lysozyme was limited to the O fractions; the Vi antigen was unchanged.

In vivo experiments showed, however, that lysozyme treatment in the presence of EDTA did not destroy the immunogenic capacity of the antigens.

[See also this *Bulletin*, 1958, v. 33, 973; 1959, v. 34, 269.] *H. J. Bensted*

D'ALESSANDRO, G. & COMES, R. Il potere immunizzante degli antigeni O, H, Vi della *S. typhi* e dell'antigene H della S. Minnesota studiato attraverso il saggio batteriemico nel topolino. [**The Immunizing Power of** *Salmonella typhi* **O, H, and Vi Antigens and of the H Antigen of Salm. Minnesota in Mice**] *Riv. Istituto Sieroterap. Ital.* 1959, Sept.–Oct., v. 34, No. 5, 330–42. [19 refs.] English summary.

This paper is a follow-up of LIPP's study of the immunizing power of typhoid vaccines by their effect in preventing bacteriaemia after a non-lethal intraperitoneal inoculation of typhoid organisms in mice [this *Bulletin*, 1958, v. 33, 607]. The authors inoculated mice which had been actively immunized with killed suspensions of *Salmonella typhi* Ty2 and O 901, and Salm. Minnesota, and by purified O, H, and Vi antigens intraperitoneally with a non-lethal dose of 10–20 millions *Salm. typhi*, or Salm. Minnesota, together with control unimmunized mice. Other mice were passively immunized with sera from rabbits immunized against one of the above antigens.

In the controls bacteriaemia was very constant and became obvious within 1 hour of inoculation with Ty2 though it was less constant with Salm. Minnesota.

The results in the immunized mice were : (1) the Vi antigen had definite protective action both active and passive; (2) the O antigen acted differently in the active and passive protection tests; in the former it was not satisfactory; (3) the H antigen did not have any active immunizing power and the results of the passive protection tests varied considerably; (4) active immunization of mice by suspensions of flagella of Salm. Minnesota similar to those of *Salm. typhi* did not give satisfactory results though 2 Salm. Minnesota antisera, 1 of whole organisms, the other of flagella had a clear protective effect on Salm. Minnesota infection though not on those with *Salm. typhi*. After absorption of O and H antibodies no protective effect was found though sera with absorbed O but still rich in

H antibodies gave reduced protection. Sera absorbed with Salm. Java had no protective effect so that in this case although the O antibodies remained, those from H were limited to the *enx* factor. Hence the protective action of the anti-flagellar serum must have been due to factor b.

The authors conclude as follows: (1) The Vi antigen of *Salm. typhi* has a definite immunizing power; (2) that of O is still not clear since it differs as regards active and passive immunization; (3) the results with the H antigens of the salmonellae are indefinite and no decision on the immunizing capacity of flagella can be made; (4) for Salm. Minnesota the protective effect of the H antigen is due to the b component.

W. K. Dunscombe

SMITH, H. W. **The Isolation of Salmonellae from the Mesenteric Lymph Nodes and Faeces of Pigs, Cattle, Sheep, Dogs and Cats and from other Organs of Poultry.** *J. Hygiene.* 1959, Sept., v. 57, No. 3, 266–73. [16 refs.]

The precise significance of the presence of salmonella organisms in the mesenteric lymph nodes of animals is not known, but the bacteriological examination of these lymph nodes has long been established as the method of choice for the demonstration of salmonellae in farm and domestic animals—particularly in pigs. From the mesenteric glands of 500 slaughtered pigs the author isolated salmonellae from 60 of the samples whereas faecal examinations of 500 market pigs yielded only 6 isolations. Compared with the results of previous surveys the isolation rate from the mesenteric glands is considerably higher; this could be partially explained by the technique employed. After appropriate treatment individual groups of lymph nodes from each pig were cultured in both selenite broth and brilliant green MacConkey broth. Some years ago the author [SMITH, this *Bulletin*, 1952, v. 27, 786] showed that both selenite broth and tetrathionate broth were unsuitable as enrichment media for the isolation of *Salmonella cholerae-suis*. Of the 60 strains of Salmonellae isolated 11 were identified as *Salm. cholerae-suis*, a significant proportion of the total, and all of these were isolated only from the brilliant green MacConkey broth cultures. The selenite broth cultures failed to reveal this organism, yet it is the commonest used enrichment medium for surveys of the type recorded here.

Of the other animals examined 4·5% of the mesenteric glands of dogs yielded salmonellae and 2·5% of the glands of cats. While it would seem that the demonstration of salmonellae in these animals is a clear indication that the animals in question had acquired these organisms as a result of consuming infected foodstuffs of varying sorts, the part they play in disseminating salmonella infections is still uncertain.

There seems to be little doubt, however, from the work of NEWELL and his colleagues [*ibid.*, 1959, v. 34, 670] on salmonella infections of pigs that some outbreaks of salmonella food-poisoning in man can be directly connected with the consumption of contaminated meat from pigs which were originally infected from feeding-stuffs (fish-meal or bone-meal) heavily contaminated with salmonella organisms.

H. J. Bensted

BÖVRE, K. & SANDBU, P. ***Salmonella*-excreting Tortoises in Oslo.** *Acta Path. et Microb. Scandinavica.* 1959, v. 46, No. 4, 339–42. [10 refs.]

"33 tortoises in Oslo have been examined for the presence of Salmonella bacteria in their excreta. From 27 tortoises Salmonella bacteria were isolated, comprising the following nineteen serological types: S. abony, S. adamstua, S. bispebjerg, S. canastel, S. durban, S. grumpensis, S. hofit, S. hull, S. kottbus, S. lethe, S. lindern, S. manhattan, S. richmond, S. rubislaw, S. salford, S. taunton, S. tel-el-kebir, S. uphill, and S. wagenia."

WINKLE, S. & ROHDE, R. Die Bedeutung von Mischinfektionen durch mehrere Salmonella-Typen für Diagnostik und Epidemiologie. [**The Diagnostic and Epidemiological Significance of Mixed Infections due to Several *Salmonella* Serotypes**] *Zent. f. Bakt.* I. Abt. Orig. 1958, v. 173, Nos. 1/2, 153–8.

An epidemiological sequel to the important earlier work of the authors' laboratory [this *Bulletin*, 1956, v. 31, 1150] on the isolation of multiple salmonella serotypes from imported fish meal has been a greater incidence of mixed salmonella infections in human subjects. The authors cite a number of instances. One was a girl with gastro-enteritis from whose stools *Salm. typhi-murium* was isolated. When specimens from contacts were examined, her brother's stools yielded Salm. Tennessee. A clearance specimen from the girl herself yielded Salm. Muenchen. This led to intensive examination of a further specimen from her from which both *Salm. typhi-murium* and Salm. Muenchen were isolated.

Another girl with clinical dysentery was found to be excreting *Salm. typhi-murium* and Salm. Tennessee and a child in a Hamburg hospital excreted 4 different salmonella serotypes at once. 3 further double infections are briefly recorded.

Circumstantial evidence and some experimental work suggested that meat from animals infected with several salmonella types through contaminated feedings stuffs had been the main vehicle of infection. The authors discuss the epidemiological complexity of episodes of this kind and the risk of discredit on reporting laboratories unless great care is taken in the examination of specimens from patients with mixed infections.

B. Moore

ROHDE, R. Neue serologische Untersuchungen über die Problematik der *Salmonella*-H-Antigene i; r und r(i). [**New Serological Investigations on the Significance of the *Salmonella* Antigens, i; r and r(i)**] *Zent. f. Bakt.* I. Abt. Orig. 1959, v. 174, Nos. 5/6, 315–26.

Salmonella Altona, with its antigenic formula (8),20; r(i); z_6 occupies an intermediate position in the Kauffmann-White scheme between Salm. Kentucky (8),20; i; z_6 and Salm. Pikine (8),20; r; z_6. The

question arises whether it merits specific rank or is merely a variant of one of the other 2 serotypes. This was investigated in two ways, *viz.* by a study of the comparability of antisera produced in rabbits with different r, i and r(i) antigens, and by slide agglutination of single colonies of Salm. Altona with absorbed sera to detect possible phase variation. Several rabbits were inoculated with each type of antigen to exclude individual minor differences in antibody response of different animals to the same antigen.

Antisera to the i flagellar antigen were prepared in 5 rabbits by inoculation of H suspensions of 5 different *Salm. typhi-murium* strains. 5 other antisera to the r flagellar antigen were prepared with 5 Salm. Heidelberg strains. In addition, antisera were prepared against the i antigen of a Salm. Kentucky strain, the r antigen of a Salm. Pikine strain and the r(i) antigen of 3 different strains of Salm. Altona. For each antiserum the immunizing suspension used was a formolized one washed off soft agar swarm plates to which antiserum to the corresponding non-specific phase had been added.

H suspensions of all 5 *Salm. typhi-murium* strains were agglutinated by the Heidelberg r antisera. With a single exception the titres obtained ranged from 1:320 to 1:5000. These suspensions were also agglutinated by the r antiserum prepared against Salm. Pikine. Similar agglutination of the H suspension of Salm. Kentucky was observed.

Cross-agglutination also occurred between the various suspensions of r antigen and i antisera, although to rather lower titres.

H suspensions of the 3 Salm. Altona strains gave considerably higher titres against both i and r antisera than the titres of cross-agglutination above described.

These initial tests with unabsorbed sera showed a serological relationship between the antigens i and r, but the flagellar antigen of Salm. Altona behaved differently from both. Further tube agglutinations were now put up with absorbed sera, *viz.* with the 5 original *Salm. typhi-murium* antisera absorbed with Salm. Heidelberg suspensions, the Heidelberg antisera after absorption with *Salm. typhi-murium* and with 2 Salm. Altona sera, one absorbed with i and the other with r suspensions. In these tests, the r and i absorbed antisera were now monospecific so far as agglutinations with *Salm. typhi-murium* and Salm. Heidelberg were concerned. Both, however, agglutinated Salm. Altona, the r antiserum virtually to its full titre.

The stability of the flagellar antigen of Salm. Altona was further confirmed by slide agglutination of 100 single colonies of Salm. Altona against the 12 absorbed sera above described. Without exception all colonies gave rapid agglutination with the r antiserum and a slower but quite definite slide agglutination with i antiserum.

The author discussed these findings with Kauffmann, and suggests that these findings are most readily explained by supposing that the flagellar antigens of *Salm. typhi-murium*, Heidelberg and Altona are complex and might be designated as follows:—

Salm. typhi-murium = i_1, i_2
Salm. Heidelberg = i_2, r
Salm. Altona = i_1, r, (i_2)

B. Moore

CALLOW, Bessie R. **A New Phage-Typing Scheme for *Salmonella typhi-murium*.** *J. Hygiene.* 1959, Sept., v. 57, No. 3, 346–59. [15 refs.]

The phage-typing of *Salmonella typhi-murium* was devised by FELIX and CALLOW when they introduced their phage-typing scheme for paratyphoid B [this *Bulletin*, 1943, v. 18, 865]. The original system served to distinguish 12 types and subtypes of *Salm. typhi-murium* and proved to be a valuable epidemiological tool. There were several indications, however, that the number of epidemiologically valid types was appreciably greater than then recognized. Miss Callow applied herself over the years to the task of developing an improved scheme for the finer differentiation of valid epidemiological types and shortly before she died she had completed the work described in this paper. The new system, to be called Scheme 2, offers advantages over the original Scheme 1 because of the larger number of types it identifies. Extended parallel tests of the two sets of typing phages on freshly isolated strains of *Salm. typhi-murium* are in progress in order to allot with precision places in Scheme 2 to types established by Scheme 1. The new scheme, which it is proposed should be substituted for the old, enabled, in the first instance, 34 types to be recognized; this number has now been increased to about 80. [A further paper on the extension of this work may be expected from the Central Enteric Reference Laboratory and Bureau where Miss Callow worked for so many years.] *H. J. Bensted*

DOOLEY, E. S. & HOLTMAN, D. F. **Effect of the Administration of Cortisone on the Response of Chicks to the Endotoxin of *Salmonella pullorum*.** *J. Bacteriology.* 1959, Oct., v. 78, No. 4, 562–6, 1 fig. [22 refs.]

Evidence was published in a previous paper (ROSS *et al.*, *J. Bacteriology*, 1956, v. 71, 521) to show that in baby chicks infected with *Salmonella pullorum* the arginase system, and consequently the Krebs ornithine cycle, is reactivated. Extending this work the authors show that both endotoxin (a Boivin type prepared from *Salm. pullorum*) and cortisone increase the excretion of N. in newly hatched chicks—non-protein nitrogen, urea, creatinine and uric acid. Urea has been shown to be arginolytic in origin in chicks and is therefore dependent on the presence of an active arginase system. Thus it would appear that endotoxin or cortisone can reactivate an enzyme system that normally exists only in the developing chick embryo.

The hypoglycaemia, normally found in chicks at 24 hours after treatment with endotoxin, was moderated by cortisone. The lethal action of endotoxin and indeed its pyretic effect were neutralized by the exhibition of cortisone. *H. J. Bensted*

SANDBU, P. **A New *Salmonella* Type: Salmonella Lindern 6, 14, 24:d:e, n, x.** *Acta Path. et Microb. Scandinavica.* 1959, v. 46, No. 4, 368.

"The author describes a new Salmonella type, *S. lindern* = 6, 14, 24: d: e, n, x, isolated from a tortoise in Oslo."

KAMPELMACHER, E. H., FIFE, Mary A. & EDWARDS, P. R. **A New *Salmonella* Type: Salmonella Utrecht.** *Antonie Van Leeuwenhoek: J. Microbiol. & Serol.* 1959, v. 25, No. 3, 286–8.

"A new *Salmonella* type isolated from Angola fish meal is called *S. utrecht*. The organism possesses an O antigen not hitherto recognized in the *Salmonella* group to which the symbol 52 is assigned. The O antigen is identical with that of Arizona O group 31. The antigenic formula of *S. utrecht* is 52:d:1,5."

EWING, W. H., JOHNSON, Jane G. & DAVIS, Betty R. **A Comparison of the *Shigella* Classification used in the U.S.S.R. with the International *Shigella* Scheme.** *Internat. Bull. Bact. Nomencl. & Taxon.* 1959, Oct. 15, v. 9, No. 4, 177–81.

The Shigella Centre in Atlanta asked for representative strains of various Shigella serotypes prevalent in U.S.S.R., and in this paper the authors present a table showing the correlation between the names used in Russia and those recommended and used by the International Enterobacteriaceae Subcommittee.

Several of the established serotypes were not included among the strains sent from the U.S.S.R.; some, such as Shiga's, Schmitz's and Sonne's bacilli are known to occur there, but others, such as *S. dysenteriae* serotypes 7, 8, 9 and 10, *S. flexneri* serotypes 1a, 3b, 4a, 4b and 5, *S. boydii* serotypes 3, 6, 8, 10, 11, 13, 14 and 15 may not occur or have been recognised.

There were some discrepancies between the serological identifications made in Russia and America.

S. T. Cowan

COOPER, G. N.; PILLOW, Jill A. **Experimental Shigellosis in Mice. I. Chronic Infection with *Shigella dysenteriae* Type 2** [COOPER]. *Australian J. Exper. Biol. & Med. Sci.* 1959, June, v. 37, pt. 3, 193–200, 3 figs. **II. Immunological Responses to *Shigella dysenteriae* Type 2 Infection** [COOPER & PILLOW]. *Ibid.*, 201–9, 1 fig. [19 refs.]

I. Chronic dysenteric infections may be produced in mice which have been pre-treated with streptomycin and erythromycin, by the oral administration of dysentery bacilli in as small a dose as 100 bacterial cells. Having established the infection by largely eliminating the normal flora it is necessary to prevent their return in order to allow the pathological process to develop by adding antibiotics to the water supplied to the mice. As a result of this treatment a chronic dysenteric condition is established in the bowel, particularly in the caecum, where there is much congestion, degeneration of the mucosa and considerable round cell infiltration. The infecting organism continues to be passed in the faeces. The condition is described as resembling the chronic dysentery of man.

The organism employed for the experiments described here was a streptomycin-resistant mutant of *Shigella dysenteriae* type 2, but FRETER, who originally described the method for the establishment of *Vibrio cholerae* infections in guineapigs [*Trop. Dis. Bull.*, 1956, v. 53, 50], was able to infect mice with members of the Flexner group [*ibid.*, 1957, v. 54, 38]. Freter, however, appears to have been content with his bacteriological results whereas the present authors provide macroscopical and microscopical evidence of the pathological process.

II. The second paper is concerned with the immunological response of the mice as measured by the haemagglutination test. Serum antibodies appeared during the second week after infection and were maximal about the 14th day, and fell off by the end of a month. Copro-antibodies appeared much earlier and were maximal about the 7th day. Further experiments indicated that the local elaboration of antibody was related to the lymphocytes in the exudate. Extracts of lymphocytes from artificially produced peritoneal exudates of infected mice were found to contain significant antibody against the infecting organism.

H. J. Bensted

PIÉCHAUD, Danielle & SZTURM-RUBINSTEN, Szymona. Pouvoir pathogène expérimental des bacilles dysentériques chez le cobaye. **[Experimental Pathogenicity of Dysentery Bacilli in Guineapigs]** *Ann. Inst. Pasteur.* 1959, Oct., v. 97, No. 4, 511–17.

This paper is concerned with the different routes of inoculation for the experimental infection of guineapigs with dysentery bacilli. These include the subcutaneous, intraperitoneal, intravaginal, intravesical, intranasal and intra-ocular routes. From the authors' experience it would appear that the ocular route is as simple as any and gives satisfactory results [see also this *Bulletin*, 1958, v. 33, 264]. Intravesical inoculations are difficult technically but the infectivity rate is said to be high; the bladder wall becomes congested and thickened and an ascending infection may develop. The subcutaneous route is unsatisfactory. Organisms inoculated intranasally remain viable for a considerable period but show no evidence of invasion. Representative examples of the shigella sub-groups A, B, C and D were inoculated into guineapigs by the various routes with results of the same order, but *Shigella sonnei*, phase 1, was highly pathogenic by the intraperitoneal route whereas phase 2 organisms of this species had a very low pathogenicity. *Sh. sonnei*, phase 1, inoculated intra-ocularly resulted in a typical kerato-conjunctivitis, but phase 2 failed to produce any lesion. [Intra-ocular inoculation of guineapigs for the experimental study of dysentery bacilli is favoured by workers in Hungary, [see SERENY, *Acta Microbiologica*, 1955, v. 28, 2931: also this *Bulletin*, 1958, v. 33, 690: 1959, v. 34, 673; for a general discussion on the eye as a portal of entry of infection, see PAPP, this *Bulletin*, 1959, v. 34, 969]. *H. J. Bensted*

SZTURM-RUBINSTEN, Szymona & PIÉCHAUD, Danielle. Sur l'utilisation du citrate dans la tribu des Escherichieae. **[Uptake of Citrate by Various Germs of the Escherichieae Tribe]** *Ann. Inst. Pasteur.* 1959, Sept., v. 97, No. 3, 360–69.

The tribe Escherichieae, according to the authors, appears to include the *E. coli*, *Aerobacter aerogenes*, Providence, Alkalescens-Dispar and *Shigella* groups and

this paper reports the examination of representative numbers of strains belonging to these groups in relation to their citrate utilization. 3 citrate media were used: Christensen, Simmons and Simmons with asparagin. The various members of the Shigella sub-groups A, B, C and D conformed to their definition and did not utilize citrate in the Christensen and Simmons media, but when the latter medium contained asparagin a significant proportion of strains of *Shigella dysenteriae*, *Sh. flexneri* and *Sh. boydii* grew and produced alkalinity in the medium. It is suggested that any shigella organism that would grow in Simmons citrate plus asparagin would produce alkalinity. With regard to the less exacting organisms it was found that 30/33 *E. coli* strains (presumably *E. coli* I and II) and 59/65 Alkalescens-Dispar strains grew in Christensen medium and would produce alkalinity in up to 3 days. The table set out below summarizes the main results of this investigation:

Utilization of Citrate in Different Media

Bacterial group.	Christensen	Simmons	Simmons plus asparagin
Providence	+	+	+
Aerobacter Aerogenes	+	+	+
E. coli	+	−	+
Alkalescens-Dispar	+	−	+
Shigella	−	−	− or +

H. J. Bensted

FLEISCHHAUER, G. Über einen Toxinnachweis bei Colibakterien mit Hilfe einer Präzipitinreaktion im Plattentest. [**The Demonstration of Toxin Production by Coli Strains by a Precipitation Reaction Plate Test**] *Zent. f. Bakt.* I. Abt. Orig. 1958, v. 173, Nos. 7/8, 551–62, 3 figs. [30 refs.]

The author applied the agar gel diffusion technique of ELEK [this *Bulletin*, 1948, v. 23, 445] to the investigation of toxin production by pathogenic *coli* strains. Broth cultures of the test organisms were streaked on to agar plates at right angles to filter paper strips soaked with high titre homologous antisera and laid on the surface of the agar. Satisfactory results depended on particular attention to 2 technical points. Firstly, the flocculation lines of antigen-antibody precipitation were obscured by luxuriant growth of the *coli* strains. This was overcome by pouring thin plates (10 ml.) of a poorly nutrient 0·5% peptone agar which checked the growth of the test strains. Secondly, only OB antisera gave satisfactory precipitation zones: pure O sera gave very little precipitation.

Considerable differences were found in the intensity of the reactions observed with coli strains of different O groups and their homologous antisera, and even between strains of the same antigenic structure. Nevertheless, serotypes particularly associated with severe infantile enteritis gave on the whole earlier and more marked flocculation lines than less pathogenic types. Flocculation lines that developed within 24 hours were always more clear-cut than those that developed later. The intensity of the reactions observed, as shown by the time of appearance of precipitation zones, is tabulated as follows for the various pathogenic *coli* strains investigated.

Type	*No. of strains showing precipitation zones after*			*Total*
	1 day	*2 days*	*Later*	
O 26 B6	15	0	0	15
O 111 B4	2	9	3	14
O 55 B5	2	11	7	20
O 86 B7	0	6	0	6
O 119 B14	1	11	16	28
O 127 B8	0	0	5	5

A few *E. coli* strains not associated with infantile enteritis were also examined. 1 such strain, of O group 11, gave strong precipitation with homologous antiserum; the others showed only a weak reaction coming up after several days.

Organisms killed by steaming also gave specific precipitation zones which were in some cases as marked as with the living organism. Similar results were obtained with endotoxin preparations of certain strains. Furthermore, when precipitation zones were excised with sterile precautions from plate tests done with living organisms, extracts of the agar containing the zone of precipitation produced erythema on subcutaneous injection into rabbits, an indication that toxic substances were involved in the reaction.

Similar techniques could be applied to the study of toxin production by shigella and salmonella strains.

B. Moore

HÖSEL, G.; NÜSKE, R. Experimentelle Studien zur Abgrenzung des Begriffes der sogenannten Coliwertigkeit. V. Mitteilung. Beziehungen zwischen Coli-Index und Colizinen [**Experimental Studies on the So-Called Evaluation of *Escherichia coli* Strains, V. Relation between the *coli* Index and Colicines**] [HÖSEL]. *Zent. f. Bakt.* I. Abt. Orig. 1958, v. 173, Nos. 1/2, 25–32. VI. Mitteilung. Gewinnung und chemische Eigenschaften des Colizins von *E. coli* SG 710 [**VI. Extraction and Chemical Characteristics of Colicines from *E. coli* SG 710**] [HÖSEL & NÜSKE]. *Ibid.*, 33–9, 5 figs. VII. Mitteilung. Biologische Wirkungen des Colizins von *E. coli* SG 710 und Eigenschaften einiger Abbauprodukte [**VII. Biological Effects of Colicines on *E. coli* SG 710 and Characteristics of Decomposition Products**] [HÖSEL & NÜSKE]. *Ibid.*, 40–44. VIII. Mitteilung (Schluss). Kaninchen-Hautteste und serologische Untersuchungen. Abschliessende Erörterung der Ergebnisse von Mitteilung I–VIII [**VIII. Skin Tests in Rabbits and Serological Studies**] [HÖSEL]. *Ibid.*, 45–9.

In these 4 further papers [for previous work, see this *Bulletin*, 1959, v. 34, 674] the authors continue the critical study of the methods used in Germany for many years to select *coli* strains for oral administration in viable form to dyspeptic patients. The main method

on which this selection was based was the determination of a "*coli index*", a measure of the capacity of the *coli* strains to outgrow typhoid bacilli in mixed culture. The fallacies of this test had been demonstrated in one of the earlier papers.

In the first of the present series of papers, the author records the capacity of the *coli* strains he studied to produce colicines active against other *coli* strains or various other bacterial groups, and also their sensitivity to colicines. The results failed to establish any essential differences in colicine production or sensitivity between pathogenic *coli* strains and those used in therapy.

A more intensive study of colicine production was done on one *coli* strain, SG 710, which has been widely used in this field in commercial preparations. Technical details of the optimal cultural conditions for colicine production by this strain are given in the second paper of the series. The colicine gave the reactions of a lipopolysaccharide-protein complex. The partially purified colicine showed activity only against other *coli* strains, apart from a few strains of *Sh. flexneri* and *Sh. sonnei*. These findings again offered no rational basis for the use of the strain SG 710 in *coli* therapy.

In the final paper of the series, the author records the results of serological grouping of the series of *coli* strains used in his studies, and of the necrotizing effect of broth cultures of these strains in the skin of the rabbit.

His general conclusion is that whatever the clinical efficacy of administering certain *coli* strains to dyspeptic patients, no valid bacteriological method for the selection of these strains is yet available. *B. Moore*

PARDEE, A. B., JACOB, F. & MONOD, J. **The Genetic Control and Cytoplasmic Expression of "Inducibility" in the Synthesis of β-galactosidase by** *E. coli*. *J. Molecular Biol.* London. 1959, June, v. 1, No. 2, 165–78, 5 figs. [32 refs.]

FRONTALI, Laura & TECCE, G. **Enzymic Conversion of L-Rhamnulose to L-Fuculose in** *Escherichia coli*. [Correspondence.] *Nature*. 1959, Oct. 24, v. 184, Suppl. No. 17, 1315.

KATAGIRI, H., TOCHIKURA, T. & SUZUKI, Y. **Microbiological Studies of** *Coli-aerogenes* **Bacteria. Part VI. The Action of Antibiotics on Bacterial Respiration and α-Ketoglutaric Acid-Fermentation.** *Bull. Agric. Chem. Soc. of Japan*. 1959, July, v. 23, No. 4, 322–32. [53 refs.]

GROSS, J. & ENGLESBERG, E. **Determination of the Order of Mutational Sites governing L-Arabinose Utilization in** *Escherichia coli* **B/r by Transduction with Phage P1bt.** *Virology*. New York. 1959, Nov., v. 9, No. 3, 314–31, 3 figs. [24 refs.]

COHN, M. & HORIBATA, K. **Inhibition by Glucose of the Induced Synthesis of the β-Galactoside-Enzyme System of** *Escherichia coli*. **Analysis of Maintenance.** *J. Bacteriology*. 1959, Nov., v. 78, No. 5, 601–12, 8 figs. & 2 diagrams. [30 refs.]

COHN, M. & HORIBATA, K. **Analysis of the Differentiation and of the Heterogeneity within a Population of** *Escherichia coli* **undergoing induced β-Galactosidase Synthesis.** *J. Bacteriology*. 1959, Nov., v. 78, No. 5, 613–23, 4 figs. [26 refs.]

COHN, M. & HORIBATA, K. **Physiology of the Inhibition by Glucose of the induced Synthesis of the β-Galactoside-Enzyme System of** *Escherichia coli*. *J. Bacteriology*. 1959, Nov., v. 78, No. 5, 624–35, 11 figs. [25 refs.]

ZAMENHOF, S., RICH, K. & DE GIOVANNI, Rosalie. **Studies on Thymine-5-Bromouracil "Exchange" in Deoxyribonucleic Acids of** *Escherichia coli*. *J. Biol. Chem.* 1959, Nov., v. 234, No. 11, 2960–64. [23 refs.]

FLAKS, J. G. & COHEN, S. S. **Virus-induced Acquisition of Metabolic Function. III. Formation and some Properties of Thymidylate Synthetase of Bacteriophage-infected** *Escherichia coli*. *J. Biol. Chem.* 1959, Nov., v. 234, No. 11, 2981–6, 2 figs. [14 refs.]

BARNER, Hazel D. & COHEN, S. S. **Virus-induced Acquisition of Metabolic Function. IV. Thymidylate Synthetase in Thymine-Requiring** *Escherichia coli* **Infected by T2 and T5 Bacteriophages.** *J. Biol. Chem.* 1959, Nov., v. 234, No. 11, 2987–91, 2 figs. [23 refs.]

ELSON, D. **Preparation and Properties of a Ribonucleoprotein isolated from** *Escherichia coli*. *Biochim. et Biophys. Acta*. 1959, Dec., v. 36, No. 2, 362–71, 6 figs. [31 refs.]

ELSON, D. **Latent Enzymic Activity of a Ribonucleoprotein isolated from** *Escherichia coli*. *Biochim. et Biophys. Acta*. 1959, Dec., v. 36, No. 2, 372–86, 8 figs. [28 refs.]

ROCHE, J., SALVATORE, G. & COVELLI, I. Sur le métabolisme des hormones thyroïdiennes par *Escherichia coli*. [**Metabolism of Thyroid Hormones by** *Escherichia coli*] *C.R. Soc. Biol.* 1959, v. 153, No. 7, 1116–19, 2 figs.

CLOSON, J., SALVATORE, G., MICHEL, R. & ROCHE, J. Dégradation de l'ester sulfurique de la 3:5:3′-triiodo-L-thyronine par les bactéries intestinale du rat. [**Degradation of the Sulphuric Ester of 3:5:3′-triiodo-L-thyronine by the Intestinal Bacteria of the Rat**] *C.R. Soc. Biol.* 1959, v. 153 No. 7, 1120–25, 2 figs. [14 refs.]

WIDRA, A. & WILBURN, S. **Electron Microscopy of Volutin Granules in** *Aerobacter aerogenes. Giorn. di Microbiol.* Naples. 1959, Apr.–June, v. 7, No. 2, 81–4, 7 figs. on pl. [12 refs.]

EDWARDS, P. R., ALTMANN, G., MORAN, Alice B. & FIFE, Mary A. **Five New Arizona Serotypes isolated in Israel (7: 29–25; 9: 22–31; 15: 24–25; 16: 29–31; 24: 24–31).** *Bull. Res. Counc. of Israel.* 1958, Dec., v. 7E, No. 4, 214–16.

"Five new Arizona types isolated in Israel (7: 29–25, 9: 22–31, 15: 24–25, 16: 29–31 and 24: 24–31) are described. All except serotype 15: 24–25, which was isolated from the stool of an infant affected with gastro-enteritis, were recovered from the intestinal tracts of apparently normal reptiles. One serotype (24: 24–31) later was isolated from the stool of an apparently healthy infant in the United States."

NOGRADY, G. **Primary Plating Medium for differentiating the Colonies of the Genus** *Proteus* **from certain other Enteric Bacteria.** *Applied Microbiol.* Baltimore. 1959, Sept., v. 7, No. 5, 314–17. [10 refs.]

This medium allows colonial differentiation of the urease-active *Proteus* organisms (*Proteus mirabilis, P. morgani, P. rettgeri* and *P. vulgaris*) at 15 hours' incubation at 37°C. from the urease-negative Enterobacteriaceae—typhoid, paratyphoid, dysentery and coli bacilli. The phosphate buffer is an important component of the medium because it fixes the surplus ammonia released from urea by the *Proteus* species. The characteristic strong alkalization is apparent only in the immediate neighbourhood of *Proteus* colonies. Swarming is completely controlled and growth of Gram-positive organisms is inhibited.

The medium is prepared as follows:

"*Solution A.* Add 15 g agar in 350 ml of distilled water to 450 ml of Sörensen's phosphate buffer mixture (0.067 M, pH 6.45). Boil until the agar is dissolved.

"*Solution B.* Add the following substances to 100 ml distilled water: 10 g peptone, 6 g yeast extract, 12.5 g lactose, 5 g sodium chloride, 2 ml of a 10 per cent solution of sodium desoxycholate, 20 ml of a 0.5 per cent solution of brom-thymol-blue (see below). Dissolved by heating. Mix solutions A and B and boil for 2 min. Add sterile distilled water to give a final volume for 1000 ml. Cool to 80°C and add aseptically the following: 5 ml of a 50 per cent solution of urea (sterilised by filtration), 10 ml of a 0.3 per cent solution of neutral-red (sterilised by autoclaving). Mix thoroughly, pour 17-ml quantities to each Petri plate. Dry for 20 min at 37 C. The colour of the medium should be light yellowish-brown. After incubation with a faecal dilution the inoculated medium turns to light orange.

"The brom-thymol-blue solution is prepared by heating 0.5 g of brom-thymol-blue in 2 ml of normal sodium hydroxide and adding 5 ml of distilled water, shake and add normal hydrochloric acid until a green colour appears. Adjust the volume to 100 ml with distilled water." *H. J. Bensted*

BÖRGER, K. Typendifferenzierung und-einteilung des Genus *Brucella.* [**Division and Type differentiation among** *Brucellae*] *Ztschr. f. Hyg. u. Infektionskr.* 1959, v. 145, No. 6, 510–28. [Numerous refs.]

The values of the different criteria recommended in differentiating the brucellae are discussed at length and the conclusion drawn that those of widely established significance are (1) growth on media containing thionin, (2) growth on media containing fuchsin, (3) production of H_2S, (4) requirement of CO_2; A agglutination with monospecific *Br. abortus* serum, M agglutination with *Br. melitensis* serum.

Taking these 6 tests into account and all the significant literature bearing on them it is found that 26 types or subtypes are differentiated. They may be designated according to presence or absence of these characters by formulae such as 2, 3, 4, A or 1, 2, A, M.

The factors responsible for the appearance of variant mutants as *e.g.* the "*melitensis*" forms isolated from cattle in England and in Schleswig-Holstein are also discussed but no definite conclusion is reached except that an extended classification of the brucellae is required. *J. W. McLeod*

VAN DRIMMELEN, G. C. **Bacteriophage Typing applied to Strains of** *Brucella* **Organisms.** [Correspondence.] *Nature.* 1959, Oct. 3, v. 184, Suppl. No. 14, 1079.

A *Brucella* phage is reported to produce plaques on 2 strains of *Br. abortus*, but not on *Br. melitensis* or *Br. suis*. It is suggested that phage typing of this group is now possible.

[Russian workers have been phage-typing *Brucella* strains, and they report that most cultures carry temperate phages which can be induced by UV light (DROZHEVKINA, this *Bulletin*, 1959, v. 34, 582).] *Janice Taverne*

WUNDT, W. Zur Frage der Antigengemeinschaften zwischen Brucellen und Bakterien anderer Gattungen. [**Antigenic Relationship between Brucellae and other Bacterial Families**] *Ztschr. f. Hyg. u. Infektionskr.* 1959, v. 145, No. 6, 556–63. [11 refs.]

The agglutination of bacteria other than the brucellae by sera of patients suffering from brucellosis has been repeatedly reported but, since the interpretation of such findings is difficult, the author has made direct observations on the agglutination of brucellae by antisera produced with *Proteus, Brucella tularensis* and with various salmonellae. Observations have also been made on the capacity of the different bacteria to absorb the agglutinins in these sera.

The general outcome of these observations is to show that there is a definite antigenic relationship between some salmonella strains and the brucellae. This is notably so with the O-antigen 30 common to Salm. Urbana and Salm. Godesberg, and the M element in the brucella antigen. This antigen common to the brucellae and certain salmonellae is also represented

to a slight extent in the *Br. tularensis* which, however, appears to be near to *Br. abortus*. Unsaturated anti-tularaemia serum was found to agglutinate *Br. tularensis* at a dilution of 1:5000, *Br. abortus* at 1:320, *Br. melitensis* at 1:160 and Salm. Urbana at 1:80.

J. W. McLeod

OLITZKI, A. L. **Studies on the Antigenic Structure of Virulent and Nonvirulent Brucellae with the Aid of the Agar Gel Precipitation Technique.** *Brit. J. Exper. Path.* 1959, Oct., v. 40, No. 5, 432–40, 19 figs. on 3 pls. [14 refs.]

Antisera from rabbits immunized with antigens derived from different strains of brucellae were tested for their precipitating power against homologous and heterologous antigens and quantitative titrations and absorption tests were carried out to determine any differences in the antigenic structure of *Brucella abortus*, *Br. melitensis* and *Br. suis*. The immune sera were prepared by injecting rabbits with a mixture of acetone-killed and acetone-dried bacteria and proprietary adjuvants. The antigens used for the agar-gel precipitation tests were obtained by subjecting the bacterial suspensions to the action of a Mickle disintegrator. The results of the experiments undertaken show that the soluble antigens of *Br. suis* revealed by OLITZKI and SALTIZEANU [this *Bulletin*, 1958, v. 33, 1179] are present in all strains of the genus *Brucella*. In order to produce all the 6 precipitation lines the immunization of the rabbits involves a long series of injections of inocula containing adjuvants. The 6 or more soluble antigens which could be demonstrated by the agar-gel precipitation method differed in their relative concentration in the extracts of the different bacterial species and their antigenicity, but no antigen was shown to be specific for a particular bacterial species.

H. J. Bensted

WAKE, A. **Some Aspects of Variations of** *Pasteurella pestis*. **I. The Third Colonial Form of** *Pasteurella pestis*. *Japanese J. Med. Sci. & Biol.* 1959, Apr., v. 12, Nos. 1/2, 39–54, 6 figs. on 2 pls. [27 refs.] **II. Comparative Study of Strain M1140 with the Third Colonial Form of** *Pasteurella pestis*. *Ibid.*, 55–69, 6 figs. on 2 pls. [26 refs.]

[An abstract of this paper appeared in the *Tropical Diseases Bulletin*, 1960, v. 57, 240.]

SKRODZKI, E. Recherches sur la pathogénèse de la tularémie. I. Sur la production de l'enzyme hyaluronidase par *P. tularensis* et autres bactéries du genre *Pasteurella*. [**Research on the Pathogenesis of Tularaemia. I. Hyaluronidase Production by** *Brucella tularensis* **and other Bacteria of the** *Pasteurella* **Genus**] *Bull. Inst. Marine Méd. Gdańsk.* 1959, v. 10, Nos. 1/2, 65–72. [10 refs.]

The author uses the turbidimetric method of estimating hyaluronidase described by KASS and SEASTONE [this *Bulletin*, 1944, v. 19, 730] with only minor modifications, using hyaluronic acid in a 1 in 4 dilution and prolonging the period of incubation of the sample with hyaluronic acid to 1 hour.

9 strains of *Brucella tularensis* of different origin and virulence were examined, the enzyme being sought in the bacterial suspensions and in autolysates. Similarly 3 strains of *Pasteurella pestis* and 3 of *P. pseudotuberculosis rodentium* were examined. No hyaluronidase was found in any of these strains, while this was clearly present in staphylococci and pneumococci used as controls. The author could not trace any reference to studies on the presence of hyaluronidase in *Br. tularensis*. As for *P. pestis*, KOROBKOVA reported the presence of hyaluronidase, using intradermal injection in the rabbit of the extract and China ink or trypan blue solution. The author thinks this reaction was not specific.

E. Agius

TOMASZUNAS, S. **Attempts at Attenuation of** *Bacterium tularense* **Strains.** *Bull. Inst. Marine Med. Gdańsk.* 1959, v. 10, Nos. 1/2, 73–82. [11 refs.]

Experiments were carried out to obtain avirulent strains and to use these as immunizing agents. These were carried out on 6 strains of *Brucella tularensis* of animal origin and 1 of human origin.

After cultivation on agar cystine glucose blood medium to which 5% anti-tularaemic guineapig serum was added, the virulence of 1 out of 2 strains, a strain originally isolated in 1953 from *Microtus arvalis*, was lost. After 35 passages, doses of 100 million and 1 milliard did not kill mice. This strain similarly passaged in the absence of antiserum retained its virulence.

Another method depended on passaging the strains on agar cystine glucose blood medium containing 2·5% agar instead of a standard 1·5% and substituting 5% sheep blood for an equal proportion of rabbit blood. After 200 daily subcultures the virulence of 6 strains was so reduced that only 5 to 15% of mice died after doses varying from 1,000 to 1 milliard bacteria, the original lethal dose having been 1 to 10 bacteria.

A third method depending on subculturing on media which contain less and less cystine, which is essential for their growth, was not very successful, eventual attenuation not being more marked than could be attributed to continued subculturing.

The virulence of the 7 strains used was tested at the beginning and the end of the experiment 12 months later. Only 1 strain had lost its virulence, which remained practically unchanged in the others.

A dose of 10,000 of the attenuated bacteria was found when tested on 140 mice, to enable 84% of them to resist 10,000 MLD of the virulent strain given 15 days later.

E. Agius

JUDITH, F. R. & WEINBERG, E. D. **Correlation of Amine Lysis with Species Differentiation in the Genus** *Bacillus*. *J. Bacteriology.* 1959, Oct., v. 78, No. 4, 485–7, 1 fig.

Amines, acridines and phenothiazines, added in low concentration to post-logarithmic phase broth cultures of *Bacillus subtilis*, had been shown to induce lysis

of the bacterial cells (Weinberg, Billman and Bords, *Exper. Cell. Res.*, 1958, v. 15, 625). The present paper extends this observation to certain other *Bacillus* species.

As might have been expected, *B. licheniformis* behaved like *B. subtilis* and cells were lysed by many of the compounds tested. On the other hand, strains of *B. megaterium, B. cereus, B. anthracis, B. circulans, B. macerans* and *B. sphaericus* were generally resistant.

S. T. Cowan

Ciucă, M., Stamatin, N., Zilişteanu, Eugenia, Nafta, I. & Angelesco, S. Comportement au contact du phage spécifique des variétés asporogènes du *B. anthracis* isolées de souches virulentes, sporogènes apparemment résistantes aux phages. [**Behaviour of the Asporogenous Variants of** *B. anthracis* **of Virulent Sporogenous Strains, apparently Phage Resistant in Contact with the Specific Phage**] *Arch. Roumaines Path. Expér. et Microbiol.* 1959, June, v. 18, No. 2, 169–73. [17 refs.]

During work on the identification of strains of *Bacillus anthracis* by their sensitivity to the phages W, γ and E, it was noticed that some strains recently isolated from cows and pigs were only apparently resistant to the phages. On subculture they produced mixed colonies of sensitive and resistant organisms. Secondary resistant colonies growing up in areas of lysis were found to be sporebearers, whereas the sensitive organisms were asporogenous.

On plates seeded with phage resistant strains the colonies were flat, blue-grey in colour and transparent, and they produced spores. Daughter colonies arising later were often raised, yellow-white in colour and opaque; on testing they were found to have lost the ability to form spores and at the same time to have become sensitive to the 3 phages. *Janice Taverne*

Brookes, P., Crathorn, A. R. & Hunter, G. D. **Site of Synthesis of the Peptide Component of the Cell Wall of** *Bacillus megaterium. Biochem. J.* 1959, Nov., v. 73, No. 3, 396–401, 3 figs. [16 refs.]

Scheibel, I. & Assandri, J. **Isolation of Toxigenic L-Phase Variants from** *Cl. tetani. Acta Path. et Microb. Scandinavica.* 1959, v. 46, No. 4, 333–8. [29 refs.]

"L-phase variants isolated by means of penicillin from four toxigenic strains of *Cl. tetani* have been shown to retain their capacity to produce tetanus toxin.

"Toxin production occurs both in the presence and in the absence of penicillin. The amount of toxin produced is of the same order of magnitude as that produced by the bacillary phase.

"Failure to obtain toxigenic L-phase variants may be due to the presence of a high percentage of non-toxigenic mutants in the mother strain."

Doak, G. O., Freedman, L. D. & Clark, J. W., Jr. **Ionic Requirements of** *Treponema pallidum.* **II. Ammonium, Lithium, Rubidium, and Cesium.** *J. Bacteriology.* 1959, Nov., v. 78, No. 5, 703–8. [12 refs.]

Faine, S. **Virulence in Leptospirae. III. Comparison of Sensitivities of Virulent and of Avirulent** *Leptospira icterohaemorrhagiae* **to Cultural Conditions.** *J. Bacteriology.* 1959, May, v. 77, No. 5, 599–603, 3 figs. [14 refs.]

The author has already described the differences he found in the behaviour *in vivo* of virulent and non-virulent strains of *Leptospira icterohaemorrhagiae* [this *Bulletin*, 1957, v. 32, 623]. This article records the fate of these strains in culture media, made approximately isotonic to body fluids, and held at 30°, 37° and occasionally 40°C.

In the first experiments small inocula of 1 virulent and 1 non-virulent strain were seeded into Korthof's medium (which contains 0·14% NaCl) and into a Korthof-saline medium with an NaCl concentration of 0·85%. After incubation for 67 hours at 30°C., 90–98% of both virulent and non-virulent strains were alive in both media. After the same time at 37°C. the virulent strain was still active in both media; the non-virulent strain survived in Korthof's medium but had died out completely in Korthof-saline.

Temperatures of 40°C. are reached in animals infected with *L. icterohaemorrhagiae* and the micro-organisms obviously survive this temperature *in vivo* since they retain their infectivity up to the death of the animal. Experimentally it was found that the virulent strain grew in Korthof's medium at 40°C. but not in Korthof-saline.

The author suggests that the relative resistance of virulent strains in conditions resembling those *in vivo* could explain their survival in infected animals.

[As regards the cultural experiments at 40°C. it should perhaps be noted that the cultures were apparently examined only after 5 days' incubation at 40°C. In guineapigs the hyperthermia lasts for only a day or two and the temperature falls to subnormal before death. In fatal human cases of Weil's disease, in which high fever may have persisted for 5 or more days, the reviewer has only rarely been able to demonstrate leptospires in sections of liver or kidney.]

J. C. Broom

Emanuel, Marie L. **The Susceptibility of Mice to North Queensland Strains of Leptospirae.** *Australian J. Exper. Biol. & Med. Sci.* 1959, Feb., v. 37, Pt. 1, 17–29, 1 fig. [23 refs.]

This work was begun originally in an effort to discover whether the inoculation of young mice with blood from suspected cases of leptospirosis (in addition to the normal routine of making blood cultures) might increase the proportion of cases from which strains of leptospires could be isolated. The culture and inoculation experiments were not, however, strictly comparable: cultures were made immediately blood was

taken from the patient whereas the mice were inoculated about 2 days later with ground-up clot. This might have allowed leptospires to die out before the time of inoculation.

Another possible source of fallacy lay in the fact (not appreciated at the beginning of the work) that a proportion of the mice developed inapparent infections. These mice showed no clinical signs but might become carriers and excretors for varying periods of time.

Further experiments were therefore carried out to determine the relative pathogenicity for mice of the 13 serotypes which are present in Queensland, and the findings are shown in the Table. From the results the author infers that there is a pattern of pathogenicity of the different serotypes which may be characteristic for the host species, and also that a particular level of pathogenicity may make an animal a good reservoir host.

[The first inference was used by Schüffner many years ago as a possible subsidiary criterion for differentiating the various serotypes of leptospires, but increased knowledge showed that strains of the same serotype varied widely in their pathogenicity (or perhaps more properly virulence) for a single host. There is, however, no doubt about the validity of the second.]

Table

Severe	Moderate to Mild	Inapparent
australis A	*icterohaemorrhagiae*	*pomona*
grippotyphosa	*canicola*	*kremastos*
	australis B	*mini*
	" Robinson "	*medanensis*
	" Esposito "	*celledoni*
	hyos	

J. C. Broom

BACTERIOPHAGES

ZELKOWITZ, L. & NOLL, H. **Reversible and Irreversible Adsorption of T1 Phage to Water-Insoluble Polar Lipids.** *Virology.* New York. 1959, Oct., v. 9, No. 2, 151–67, 4 figs. [14 refs.]

Like some animal viruses, phage T1 was found to adsorb to columns of cholesterol [this *Bulletin*, 1959, v. 34, 687], the efficiency of adsorption depending upon the capacity of the column and the rate of equilibration. The kinetics of adsorption were determined. Infectious phage was recovered quantitatively by breaking up the column and resuspending the cholesterol. T1 was not eluted by washing the column with solutions of increasing ionic strength, but it was eluted, with some purification, by ether-water partitioning.

When antiserum was passed through a column saturated with phage, 88% of homologous neutralizing antibody was specifically removed. Neither protein nor antibody were removed by control columns without phage. It appears likely that T1 adsorbs to cholesterol by its head, leaving the tail free to reduce the blocking power of the serum.

It is suggested however, that ether-resistant lipophilic components of the phage surface also may be concerned in the adsorption of phage to the bacterial host. Cell wall extracts of *Escherichia coli* which retain the phage receptors are known to have a high lipid content. Although the adsorption of T1 to neutral and acid lipids (cholesterol and stearic acid) was found to be reversible, adsorption to the positively charged hexadecylamine was irreversible. The authors propose that T1 adsorbs to this substance by its tail, with concurrent loss of infectivity, and that hexadecylamine may be a valuable chemical model of the cellular receptor. It makes unnecessary the hypothesis that adsorption takes place in 2 stages, the first being reversible and ionic, and the second involving an enzyme. (TOLMACH, *Advances in Virus Res.*, 1957, v. 4, 63).

Janice Taverne

CAMPBELL, Janet G. **Anamnestic Responses in Rabbits immunized with Two Related Bacterial Viruses.** *Australian J. Exper. Biol. & Med. Sci.* 1959, June, v. 37, Pt. 3, 245–52, 5 figs. [15 refs.]

The antibody response to the related phages T2 and T4 was studied in 8 rabbits, 4 of which were given the homologous and 4 the heterologous phage as a secondary stimulus. In every case, the titre of neutralizing antibody against both phages rose logarithmically, reaching a maximum on the seventh day after the booster dose. Injection of T2 alone resulted in the production of antibody of the same order of activity against T2 and T4, but injection of T4 alone produced only small amounts of T2 antibody compared with T4; in each case injection of the heterologous phage caused a rise in antibody to that phage.

The sera were absorbed with bacteria coated with phage and antibody levels again determined. No evidence was obtained of an increase in specific homologous antibody after stimulus with the heterologous antigen (*i.e.* of a true anamnestic reaction). Instead, there was an increase in the cross-reacting antibody which had appeared on primary immunization.

Janice Taverne

KAY, D. **The Inhibition of Bacteriophage Multiplication by Proflavine and its Reversal by certain Polyamines.** *Biochem. J.* 1959, Sept., v. 73, No. 1, 149–54, 6 figs. [14 refs.]

The polyamines, putrescine and spermidine, have been detected in phages T2 and T4 and are thought to be the minor components which accompany phage DNA into the bacterium upon infection [AMES *et al.*, *Science*, 1958, v. 127, 814; this *Bulletin*, 1958, v. 33, (707)]. Phage 3, isolated from sewage, resembles T4 [*ibid.*, 1958, v. 33, 283] and when intact phage or a trichloracetic acid extract was subjected to paper

chromatography, it too was found to contain putrescine and spermidine. The structural similarity of these polyamines to proflavine suggested that proflavine may block the formation of infective phage, producing "doughnuts" or empty heads, by interference with the polyamines.

Phage 3 was grown in *Escherichia coli* cells in the presence of (a) proflavine only, and (b) proflavine and different concentrations of various polyamines, and the burst size determined. The reduction in burst size caused by proflavine was reversed by putrescine, and to a lesser extent by spermidine. Of the polyamines absent from the phage, agmatine showed the greatest reversing power. None of these substances entirely reversed the action of proflavine, and spermidine itself, in high concentration, was found to inhibit phage multiplication. From experiments in which these agents were added to cultures at different times during the phage growth cycle, it was deduced that proflavine blocks a late stage in maturation. At lower but not at higher concentrations of proflavine, the polyamines had a competitive effect.

The author suggests that the polyamines may both neutralize acidic groups in phage DNA and maintain important internucleotide distances. Then, only when the correct polyamine-DNA relationship is established can the DNA be orientated to form the core of the phage head. Proflavine may interfere with this process by displacing the naturally occurring polyamines.

Janice Taverne

FREESE, E. **The Specific Mutagenic Effect of Base Analogues on Phage T4.** *J. Molecular Biol.* London. 1959, June, v. 1, No. 2, 87–105, 6 figs. [12 refs.]

STENT, G. S., SATO, G. H. & JERNE, N. K. **Dispersal of the Parental Nucleic Acid of Bacteriophage T4 among its Progeny.** *J. Molecular Biol.* London. 1959, June, v. 1, No. 2, 134–46, 2 figs. [27 refs.]

KRIEG, D. R. **Induced Reversion of T4r_{II} Mutants by Ultraviolet Irradiation of Extracellular Phage.** *Virology.* New York. 1959, Oct., v. 9, No. 2, 215–27. [31 refs.]

Previously, UV-irradiation of free phage had not been shown to induce mutation, although irradiation of intracellular phage is well known to do so. This paper reports the results of carefully designed experiments which indicate that UV-irradiation of extracellular phage does increase its mutation rate.

The system used, the reversion of T4 r II mutants, was chosen for a number of reasons: phage T4 because it does not undergo UV restoration, by recombination with host material, when plated on unirradiated bacteria; mutants of the r II region because they can be scored by as sensitive a technique as any known, and the system is not complicated by phenotypic mixing. Not only the survivors, but the progeny of irradiated phage were examined, to increase the chance of detecting mutants arising during phage multiplication. Phage was plated under conditions allowing multiplicity reactivation, so that higher UV doses could be used, and mutant genes recovered from inactivated as well as viable phage particles.

It was found that r II wild type reversions occurred much more frequently among the progeny of irradiated phage than among the progeny of unirradiated controls. Reconstruction experiments showed that these results reflected UV-induced reverse mutations, and not the preferential growth or survival of r II wild type particles under these conditions. *Janice Taverne*

WASSERMANN, F. E. **The Inheritance of Host Range Differences between the Related Phages T5 and PB.** *Virology.* New York. 1959, Nov., v. 9, No. 3, 425–36. [15 refs.]

OTSUJI, N., SEKIGUCHI, M., IIJIMA, T. & TAKAGI, Y. **Induction of Phage Formation in the Lysogenic *Escherichia coli* K-12 by Mitomycin C.** [Correspondence.] *Nature.* 1959, Oct. 3, v. 184, Suppl. No. 14, 1079–80, 2 figs.

Mitomycin C, a new antibiotic, has been shown to inhibit the synthesis of DNA in bacteria, though DNA synthesis is restored upon infection with T2 [SEKIGUCHI and TAKAGI, *Nature*, 1959, v. 183, 1134; this *Bulletin* 1959, v. 34, (812)]. When it was added to a culture of *Escherichia coli* K-12 the cells continued to grow for a time but then lysed. The growth curve resembled those obtained for lysogenic bacteria after induction with appropriate doses of UV light. Lysis was prevented by chloramphenicol. A non-lysogenic strain was not lysed by any amount of mitomycin C.

It was found that lysis was caused by the release of the lysogenic phage λ from the cells of *E. coli* K-12 after a latent period, and that mitomycin C thus induces the development of the phage in the same way as UV irradiation. *Janice Taverne*

CAMPBELL, A. **Ordering of Genetic Sites in Bacteriophage λ by the Use of Galactose-Transducing Defective Phages.** *Virology.* New York. 1959, Nov., v. 9, No. 3, 293–305. [19 refs.]

THOMAS, R. **Effects of Chloramphenicol on Genetic Replication in Bacteriophage λ.** *Virology.* New York. 1959, Oct., v. 9, No. 2, 275–89, 4 figs. [2 refs.]

If chloramphenicol is present at the time of infection of phage T2, phage DNA synthesis is inhibited, as well as protein synthesis. If, however, it is added several minutes after infection, phage DNA synthesis is not inhibited and genetic material accumulates. This can later be incorporated into mature phage particles forming after the removal of chloramphenicol [this *Bulletin*, 1957, v. 32, 692]. The early step in DNA synthesis which is sensitive to chloramphenicol and presumably involves protein synthesis, could arise

because the change from synthesis of bacterial to phage DNA depends on either (1) the synthesis of an enzyme, or some other metabolic or structural readjustment, or (2) the transfer of genetic information from phage DNA to a protein-containing structure. If this last hypothesis is right, then, if bacteria are infected with one phage and chloramphenicol is added before superinfection with a second phage, DNA from the first phage only should accumulate.

It was found that when *Escherichia coli* W 3110 was infected with a virulent mutant of λ having a wild type host range (h+), and chloramphenicol was added before superinfection with a host range mutant (h), the genetic pools of both phages increased equally during exposure to chloramphenicol. The progeny released after removal of the inhibitor consisted of h and h+ in the same ratio as in the parents. Thus although there is an early step in the DNA synthesis of phage λ, which, like T2, is sensitive to chloramphenicol, it does not seem to arise from the dependence of DNA synthesis on the preliminary recoding of information by a process involving protein synthesis.

Janice Taverne

KOZINSKI, A. W. & SZYBALSKI, W. **Dispersive Transfer of the Parental DNA Molecule to the Progeny of Phage φX-174.** *Virology.* New York. 1959, Oct., v. 9, No. 2, 260–74, 4 figs. [19 refs.]

During replication of the double-stranded DNA helix, it is possible that (1) the duplex molecule remains whole, (2) the 2 strands separate, but the identity of each strand is preserved, or (3) the DNA breaks up into its component parts which mix with those of the newly synthesized DNA. Experiments designed to distinguish between the last 2 possibilities are reported here.

Phage φX-174 was used because it appears to contain a single strand of DNA only (SINSHEIMER, *J. Molecular Biol.*, 1959, v. 1, 43). Parental and progeny phage DNA were distinguished by labelling with 5-bromodeoxyuridine, which replaces thymidine in the DNA molecule. Phage with labelled DNA was heavier than normal phage. It was recognized either by its position after centrifugation in a density gradient of caesium chloride, or by its greater sensitivity to inactivation by UV light.

In an experiment in which bacteria were infected with "heavy" labelled phage, none of the progeny were found to be "heavy" by centrifugation. Again, when the experiment was repeated the other way round and "light" unlabelled parental phage multiplied in cells in a labelled medium, none of the progeny were found to be "light", as shown by their sensitivity to UV rays. Theoretically, less than 1 particle in 1,000 of the other kind could have been detected.

It seems therefore, that the integrity of the DNA molecule is lost in replication, and the label is scattered among the nucleotides in the DNA pool. That is, the mode of replication of the single molecule of DNA of phage φX-174 is *dispersive.* *Janice Taverne*

TESSMAN, I. **Mutagenesis in Phages φX174 and T4 and Properties of the Genetic Material.** *Virology.* New York. 1959, Nov., v. 9, No. 3, 375–85, 4 figs. [17 refs.]

SINSHEIMER, R. L. **Purification and Properties of Bacteriophage φX174.** *J. Molecular Biol.* London. 1959, Apr., v. 1, No. 1, 37–42, 2 pls. & 2 figs. [28 refs.]

SINSHEIMER, R. L. **A Single-Stranded Deoxyribonucleic Acid from Bacteriophage φX174.** *J. Molecular Biol.* London. 1959, Apr., v. 1, No. 1, 43–53, 8 figs. & 1 pl. [46 refs.]

HALL, C. E., MACLEAN, Elizabeth C. & TESSMAN, I. **Structure and Dimensions of Bacteriophage φX174 from Electron Microscopy.** [Correspondence.] *J. Molecular Biol.* London. 1959, June, v. 1, No. 2, 192–4, 1 pl.

HARADA, K. **Studies on Directed Variation of *Salmonella* 0–34 by Bacteriophage. I. Antigenic Transformation by Bacterial Autolysate and Antiserum.** *Japanese J. Microbiol.* 1959, Jan., v. 3, No. 1, 53–60. [18 refs.] **2. Antigen-Transforming Agent in Bacteria of Group E_3.** *Ibid.*, 61–9, 1 fig. [16 refs.]

See also p. 290, TAKEYA, YOSHIMURA, YAMAURA & TODA, **Studies on the Biologic Properties of Mycobacteriophage.**

VIRUSES

The order in this section is:—**General; Psittacosis-Lymphogranuloma group; Pox group; Respiratory group; Enteric group; Arthropod-borne group; Others.**

SMITH, K. M. **Recent Work on the Electron Microscopy of Viruses.** *Nature.* 1959, Nov. 7, v. 184, 1440–45, 4 figs. [30 refs.]

This paper is the substance of a University of London Special Lecture. It begins with summaries of the techniques ordinarily used in electron microscopy. Shadow casting with metals is described, together with methods of cutting ultra-thin sections and the media used for embedding them. The most useful stains are listed, from the commonly used osmic acid to the newer and very successful phosphotungstic acid. Substances used for supporting films are mentioned and the technique of making carbon replicas is described. The advantages of fixing viruses and sections by freeze-drying to prevent shrinkage and distortion are discussed, with methods for doing so.

Examples of some of the knowledge acquired by the

application of these techniques follow. Much has been discovered about the size and shape of virus particles. Some viruses have been shown to be built up of identical protein sub-units, regularly arranged. An adenovirus particle, for instance, has icosahedral symmetry, each triangular facet having 6 sub-units. Little is known about the internal structure of viruses, though empty particles can be distinguished, but some work has been done by degrading the particles chemically, step by step, on the microscope grid. In this way, digestion of the outer protein component of influenza virus was found to uncover a nucleoprotein ring. Microscopy has provided information on the multiplication of viruses, particularly of bacteriophages, and on the behaviour of viruses within the cell. Herpes simplex virus, for example, has been shown to multiply in the nucleus, and stages in its development have been followed. Similar work has been done with insect viruses. Many viruses have now been obtained in crystalline form. Plant viruses were the first to be purified sufficiently to form crystals, but now poliomyelitis virus and the *Tipula* iridescent virus have been found to show the same phenomenon. Adenoviruses and the virus of herpes simplex have been seen in the form of crystalline inclusions within the cell.

The author ends with a paragraph on latent viruses, which are known to infect plant and mammalian, as well as insect cells. So far, however, attempts to see latent viruses within the cell, under the electron microscope, have proved disappointing. *Janice Taverne*

Ianconescu, M. & Aderca, I. Susceptibilitatea culturilor de celule embrionare umane, obținute prin tripsinizare, la infecția cu unele virusuri. [**Susceptibility of Cultures of Human Embryo Cells obtained by Trypsinization to Infection with Various Viruses**] *Studii si Cercetări Inframicrobiol.* 1959, v. 10, No. 2, 193–8. French summary.

The authors have investigated the growth of a number of viruses in trypsinized primary explant cultures of human embryonic tissue. The Mahoney, Brunhilde, MEF 1 and Saukett strains of poliomyelitis virus produced a cytopathic effect after 24 hours and multiplied to titres of 10^6–10^7. With 12 types of ECHO virus a cytopathic effect was observed after 24 hours, with 5 types of APC virus after 4–5 days, and with vaccinia virus after 48 hours. 4 strains of herpes virus produced a cytopathic effect after 24–48 hours and multiplied to titres of 10^5–$10^{5\cdot5}$. No evidence of the multiplication of influenza, mumps or rabies viruses could be obtained. *D. J. Bauer*

Volkert, M.; Matthiesen, M. **Ornithosis-Related Antigens from *B. anitratum* in the Serological Diagnosis of Ornithosis. I.** [Volkert & Matthiesen]. *Acta Path. et Microb. Scandinavica.* 1958, v. 44, No. 3, 278–86. [15 refs.] **II.** [Matthiesen & Volkert]. *Ibid.*, No. 4, 440–46.

I. In the first paper the authors show that complement-fixation tests made with 5 selected ornithosis sera and antigenic preparations of *Bacterium anitratum* revealed a soluble ornithosis-related antigen in 22 out of the 30 strains of the bacterium examined. Titration of these 22 antigens with the same sera revealed considerable differences in antigenic quality and a combined antigen was prepared from 2 strains giving the widest range of activity. Tested with 4 of the 5 selected ornithosis sera it gave complement-fixation with all 4. 172 ornithosis-positive sera were examined in the complement-fixation test with this "mixed" anitratum antigen; 165 gave a positive result. The titres were the same as with ornithosis antigen in 147, greater with ornithosis antigen in 11 and less in 7. Of 500 ornithosis-negative sera from various clinical states (mostly fevers of unknown origin, a few pneumonia, some severe sepsis) 16 fixed complement with the anitratum antigen.

II. The absorption experiments described in the second paper in which washed boiled bacteria were used for absorbing the sera, gave support to the conclusion that certain strains of *Bacterium anitratum* contain an ornithosis-related antigen. *S. P. Bedson*

Kaplan, C. & Valentine, R. C. **The Infectivity of Purified and Partially Purified Preparations of Vaccinia and Cowpox Viruses.** *J. General Microbiol.* 1959, June, v. 20, No. 3, 612–19, 4 figs. on pl. [16 refs.]

Vaccinia and cowpox viruses were extracted from infected rabbit skin, chorioallantoic membranes and tissue culture fluid and purified by treatment with Arcton 63, differential centrifugation, trypsin treatment or flocculation with *M* sodium chloride solution. The number of virus particles in the fluid preparations was counted by spraying with marker spheres of polystyrene latex and examination in the electron microscope, and the infectivity titre was determined by pock counting. In the examination of vaccinia virus purified from rabbit skin by centrifugation the ratio of particles to infective units ranged from 7 to 129, and from 1·2 to 10·7 in 7 preparations purified by flocculation. Ratios of 3·4 to 22·5 were found in 7 preparations from chick embryos purified by centrifugation alone or combined with trypsinization, and from 10·9 to 18·6 in 5 preparations from tissue culture fluid purified by centrifugation. Values of 54·5 to 150 were observed in 3 preparations of cowpox virus. *D. J. Bauer*

Wheeler, C. E. & Canby, C. M. **Effect of Temperature on the Growth Curves of Herpes Simplex Virus in Tissue Culture.** *J. Immunology.* 1959, Oct., v. 83, No. 4, 392–6, 5 figs. [17 refs.]

The amount of herpes simplex virus released into the medium after infected HeLa cells had been incubated for 3 days at different temperatures was the subject of a previous paper [Wheeler, this *Bulletin* 1959, v. 34, 499]. Growth curves for intra- and extracellular virus at the different temperatures have now been obtained. Virus with the highest titre was obtained at 35°C.; it appeared in the cells sooner than at other temperatures and large amounts were released from the cells. At 40°C. virus was detected in the

cells 1–2 hours later, but there was less of it and it was not released into the culture medium. At 30°C. only small amounts of intra-cellular virus were found after a latent period twice as long as at 35°C. No virus multiplication occurred at 25°C., although the cells looked healthy. Why intra-cellular virus formed at 30°C. and 40°C. is not liberated from the cells is being investigated. *Janice Taverne*

OSTERHOUT, S. & TAMM, I. **Measurement of Herpes Simplex Virus by the Plaque Technique in Human Amnion Cells.** *J. Immunology.* 1959, Oct., v. 83, No. 4, 442–7, 3 figs. [11 refs.]

Primary cultures of human amnion cells were grown in monolayers in 2 oz. prescription bottles, infected with herpes simplex virus and overlaid with molten agar. After 4 days' incubation at 37°C., plaques 3–4 mm. in diameter were seen. The number of plaques was directly proportional to virus concentration. The rate of adsorption of virus was similar to rates found for HeLa cells and rabbit kidney cells. Results were highly reproducible in cultures from the same amnion, and the system was about twice as sensitive as the chick chorioallantoic membrane to virus adapted to amnion cells.

The sensitivity of cultures from different amnions to the same stock of virus was found to vary enormously. It is not known why. Cultures became progressively less sensitive with time, but this could be overcome by inoculating them routinely at the same age. It is recommended that several amnions should be pooled when setting up cultures, to reduce the variability, and that a stock virus should be included in every titration to provide a standard by which results can be corrected for any variations in sensitivity which may still occur. *Janice Taverne*

MORGAN, C., ROSE, H. M., HOLDEN, Margaret & JONES, Ellyn P. **Electron Microscopic Observations on the Development of Herpes Simplex Virus.** *J. Exper. Med.* 1959, Oct. 1, v. 110, No. 4, 643–56, 30 figs. on 19 pls. [34 refs.]

Electron microscope studies on the growth of the HR strain of herpes simplex virus in cells of the chick chorioallantoic membrane had suggested that the virus originated in the cell nucleus as a particle with a central core and a single limiting membrane, but that it matured and acquired a second membrane in the cytoplasm [MORGAN *et al.*, this *Bulletin*, 1955, v. 30, 179]. By contrast, other strains of virus seemed to complete their development within the nucleus [*ibid.*, 1955, v. 30, 822; 1959, v. 34, 175]. This paper describes and illustrates with many clear electron micrographs, the development of another strain (JM) of herpes virus, in cultures of HeLa and human amnion cells.

Particles of the JM strain, like adenoviruses, have a crystalline orientation within the cell. The first particles which can be associated with virus appear in the nucleus as aggregates of uniform granules with a diameter of about 200 mμ, readily distinguishable from the reticular, marginated chromatin. Crystals of virus extend into these granular areas in a way suggesting that the granules are a template site at which the various components of the virus particles are arranged spatially, to present the appearance of a central body surrounded by a single membrane. This process seems to be quick. Presumably only if large numbers of particles were formed at the same time would they crystallize before being dispersed, and this would account for the few developmental forms and incomplete membranes seen. Virus particles with double membranes were seen within the nucleus, not in the crystals but scattered at random, suggesting that the second and outer membrane was acquired at another site. Complete virus particles appeared to enter the cytoplasm without the nucleus rupturing, a new nuclear membrane being laid down behind the virus. Virus particles within the cytoplasm were found in walled vacuoles, probably bound by extrusions of the nuclear membrane. These sacs seemed to rupture at the surface of the cell to extrude virus without lysing the cell.

In the light of these results the authors have reviewed their earlier work on the HR strain, and they conclude that the development of herpes simplex virus is completed within the nucleus. *Janice Taverne*

YOSHINO, K., TANIGUCHI, H. & TANIGUCHI, S. **Titration of Herpes Simplex Virus in One-Day Eggs by an Improved Inoculation Method and its Application to the Serum Neutralization Test.** *J. Immunology.* 1959, Sept., v. 83, No. 3, 246–51, 2 figs. [18 refs.]

The results of titrations of herpes simplex virus done (a) in the yolk sacs of 1-day-old embryonated eggs, (b) in the yolk sacs of 3–5-day eggs, and (c) on the chorioallantoic membranes (CAM) of 11–13-day eggs, were compared. 1-day-old eggs were inoculated by making a hole in the shell over the blastoderm, turning the egg slightly (through ¼ right angle) on its long axis and leaving it for an hour; then the blastoderm no longer lay beneath the hole but had risen to the uppermost part of the egg, and the injection of virus through the hole did not damage the blastoderm non-specifically. Results were read after 4 days' incubation. The non-specific death rate was constant throughout the year, and allowance was made for it. The results were obtained sooner than those of yolk sac titrations in older eggs, and the method was as sensitive. However, both these methods were 10 times less sensitive than pock counts on the CAM.

The chief advantage of the method is that 1-day eggs are always available; also the short titration period compares favourably with the 10–12 days required by other yolk sac methods. "The 1-day egg technique was thought to be of special value in the serum neutralization test because of the technical ease, quick determination of titer and constancy of result, when a constant virus-varying serum system was utilized, the virus-serum mixture was incubated at 37°C. for 1 hr and five eggs were used per dilution."

[See also this *Bulletin*, 1958, v. 33, 709.] *Janice Taverne*

PORTOCALĂ, R., BOERU, V. & SAMUEL, J. Reproducerea virusului gripal cu ajutorul acidului ribonucleic virotic purificat. [**Reproduction of Influenza Virus from Purified Virus Ribonucleic Acid**] *Studii si Cercetări Inframicrobiol.* 1959, v. 10, No. 1, 51–7. [12 refs.] French summary.

A preparation of type A influenza virus concentrated from allantoic fluid by red cell adsorption and elution was extracted 3 times with ether in order to remove lipids, and the ribonucleic acid was then extracted by the phenol method of GIERER and SCHRAMM (*Nature*, 1956, v. 177, 702). Inoculation of the ribonucleic acid preparation into the allantoic cavity was followed by the production of influenza virus, which attained a haemagglutinin titre of 1,280–2,560 after 38 hours. The type A strain from which the ribonucleic acid was prepared contained an antigen of A-prime type, but this antigen could not be found in the virus which appeared after the inoculation of ribonucleic acid. Examination in the electron microscope showed that the new virus differed from the original strain in being filamentous.

D. J. Bauer

TYRRELL, D. A. J. **Interferon produced by Cultures of Calf Kidney Cells.** [Correspondence.] *Nature.* 1959, Aug. 8, v. 184, Suppl. No. 7, 452–3, 1 fig.

Calf kidney cells in tissue cultures were shown to support the multiplication of influenza A virus for periods up to 3 months with alternate rises and falls in virus titre. Such cultures were resistant to superinfection with Sendai virus and the mechanism of this resistance was shown to be dependent on interferon.

It was further observed that interferon preparations obtained in calf kidney cultures or in chick chorioallantoic membranes showed higher activity when tested in the cell type in which the interferon was formed.

H. G. Pereira

HARBOE, A. & REENAAS, R. **The Course of Antibody Development to Influenza A_2 Strains, Examined in the Haemagglutination Inhibition Test with Viruses of Different Sensitivity to Homologous Antibody.** *Acta Path. et Microb. Scandinavica.* 1959, v. 46, No. 3, 266–72. [19 refs.]

Antibody response of ferrets infected with Asian influenza virus was followed by haemagglutination-inhibition (HI) tests performed against Asian strains of varying sensitivity to antibody. The rate of antibody formation varied according to the virus used for HI titrations, being higher with sensitive than with insensitive strains. This difference was not observed in responses to a second antigenic stimulus. The results were independent of the sensitivity of the immunizing virus. Similar results were obtained with rabbits.

Possible applications of these findings to the study of human influenza are discussed. *H. G. Pereira*

GAUSH, C. R. & YOUNGNER, J. S. **A Tissue Culture Color Test for measuring Influenza Virus and Antibody.** *Proc. Soc. Exper. Biol. & Med.* 1959, Aug.–Sept., v. 101, No. 4, 853–6, 2 figs.

A colour test based on increased *p*H values observed in monkey kidney cultures infected with influenza viruses was developed for the titrations of virus infectivity and of virus neutralizing antibody. Infectivity titres determined by the colour test were lower than those obtained by the roller tube method with a mean difference of 0·56 $\log_{10}$ units. Serum neutralization tests gave specific results showing clear distinctions between Swine, A, A prime, Asian and B strains. Antibody rises were demonstrated by the colour test in paired sera from patients with influenza. Antibody titres determined by the colour test and by haemagglutination-inhibition were significantly correlated.

H. G. Pereira

MALCHAIR, R. Effets de l'actinomycetine sur le virus grippal. [**The Effects of Actinomycetin on Influenza Virus**] *Giorn. di Microbiol. Naples.* 1958, May–June, v. 5, No. 3, 137–57, 1 fig. [36 refs.]

Incubation of human group O red cells with a solution of actinomycetin for 2 hours at 37°C. brought about a marked reduction in the titre of haemagglutination produced by influenza and Newcastle disease viruses, and a similar effect was observed when influenza virus preparations were treated with actinomycetin for 5 hours and then titrated with normal red cells. Treatment with actinomycetin for 5 hours at 37°C. also abolished the infectivity of the PR8 strain of influenza virus, and when administered to mice by atomization 30 minutes before intranasal infection some prolongation of survival time was observed.

D. J. Bauer

FRITZE, E. & WORATZ, H. Zur Bindung von Grippe- und Mumpsvirus sowie des Endotoxins aus *S. abortus equi* an menschliche Erythrozyten. [**Adsorption of Influenza and Mumps Virus and of *S. abortus equi* Endotoxin in Human Erythrocytes**] *Ztschr. f. Immunitätsf. u. Exper. Therap.* 1959, Oct.–Nov., v. 118, Nos. 4/5, 368–83. [30 refs.]

The English summary appended to the paper is as follows:—

"The electrophoretic migration velocity of human erythrocytes is diminished after the fixation of influenza B or mumps virus. Inactivated viruses do not produce this phenomenon. S. abortus equi endotoxin is a lipopolysaccharid that can be adsorbed in the surface of human erythrocytes. Red blood cells, treated this way, will become agglutinable by specific antibacterial immune sera. The affinity of the endotoxin to be adsorbed by the erythrocytes is increased by pretreatment with NaOH. The electrophoretic migration velocity is not altered by the adsorption of the lipopolysaccharid. Only after prolonged storage it is diminished, perhaps by the influence of lipopolysaccharid fractions of a low initial cell affinity.

"The adsorption in erythrocytes of influenza B and

mumps virus is not altered by pretreatment with bacterial endotoxin. The migration velocity is diminished, the virus hemagglutination not altered. It is concluded that different cell surface receptors account for the adsorption of viruses and for lipopolysaccharids activated by NaOH treatment respectively.

"Influenza as well as mumps viruses inactivated at 56°C do no longer alter the electrophoretic migration velocity of erythrocytes. Inactivation destroys the enzymatic mechanism of adsorption."

ATHANASIU, P., PETRESCU, A. & POPA, M. Modificările biologice ale unei tulpini de virus gripal cultivată pe soareci albi în cursul infecției cu virus rabic de stradă. [**Biological Modifications of a Strain of Influenza Virus Passaged in Mice Infected with Street Rabies Virus**] *Studii si Cercetări Inframicrobiol.* 1959, v. 10, No. 1, 77–88, 3 figs. [11 refs.] French summary.

A strain of type A influenza virus containing an A-prime antigenic component was passaged intranasally 4 times in normal mice and also 70 times in mice which had received a subcutaneous injection of less than 1 LD50 of street rabies virus 4 to 7 days previously. The titre of the virus fell from 3·8 to 1·0 log unit after 4 passages in the normal mice, but rose to 5·1 log units during passage in the presence of rabies virus.

D. J. Bauer

BOYER, Georgiana S., DENNY, F. W., Jr. & GINSBERG, H. S. **Sequential Cellular Changes produced by Types 5 and 7 Adenoviruses in HeLa Cells and in Human Amniotic Cells. Cytological Studies aided by Fluorescein-Labelled Antibody.** *J. Exper. Med.* 1959, Nov. 1, v. 110, No. 5, 827–44, 30 figs. on 6 pls. [28 refs.]

Cytological alterations associated with infection of HeLa cells and human amnion cells by adenovirus types 5 and 7 were studied by light microscopy of fixed and stained preparations, by phase contrast microscopy of living cells and by the fluorescent antibody technique. The changes caused by several strains of adenovirus type 5 were in many ways identical with those described by the same workers [this *Bulletin*, 1957, v. 32, 818] for adenovirus types 1 and 2 whereas the cellular lesions caused by type 7 strains were similar to those of types 3 and 4. Infection by some type 5 strains gave rise to characteristic Feulgen-negative bar-like crystals which were not stained by the sera used for the fluorescent antibody technique.

The changes observed following adenovirus infection of HeLa or amnion cells were essentially identical.

[See also BARSKI and CORNEFERT, this *Bulletin*, 1958, v. 33, 996.] *H. G. Pereira*

HORNE, R. W., BRENNER, S., WATERSON, A. P. & WILDY, P. **The Icosahedral Form of an Adenovirus.** [Correspondence.] *J. Molecular Biol.* London. 1959, Apr., v. 1, No. 1, 84–6, 3 pls.

GREEN, M. **Biochemical Studies on Adenovirus Multiplication. I. Stimulation of Phosphorus Incorporation into Deoxyribonucleic Acid and Ribonucleic Acid.** *Virology.* New York. 1959, Nov., v. 9, No. 3, 343–58, 1 fig. [34 refs.]

KAPSENBERG, Jacoba G. **Relationship of Infectious Canine Hepatitis Virus to Human Adenovirus.** *Proc. Soc. Exper. Biol. & Med.* 1959, Aug.–Sept., v. 101, No. 4, 611–14. [15 refs.]

The cytopathic effect of infectious canine hepatitis virus in dog kidney cells was shown to resemble closely that of adenovirus types 3, 4 and 7 in cultures of human cells. A complement-fixing antigen reacting with adenovirus antibodies was detected in canine hepatitis antigens prepared from infected liver tissue or kidney tissue cultures. Canine hepatitis virus was found to be non-pathogenic for common laboratory animals and to be ether resistant.

It is suggested that canine hepatitis virus should be included as a member of the adenovirus group.

H. G. Pereira

PETERSEN, K. B. **Studies on the Properties of a New Cytopathogenic and Hemagglutinating Virus (Copenhagen 222).** *Acta Path. et Microb. Scandinavica.* 1959, v. 45, No. 2, 213–24, 2 figs. [21 refs.]

A virus isolated from the throat of a child with clinical influenza was slightly cytopathic for monkey kidney tissue cultures, but was shown to multiply in them and in human kidney cells by a rise in haemagglutinin. The virus, Copenhagen 222, shared many properties with the main group of myxoviruses. It agglutinated red cells from many species of animals; it caused an inapparent infection in ferrets, monkeys and hamsters; it did not grow in HeLa cells or in human amnion cells, and was inactivated by ether. But unlike the influenza viruses it did not grow in embryonated eggs or in mice. No serological relationship with influenza A, B or C, or with Newcastle disease or CA virus was demonstrable by complement-fixation or haemagglutination-inhibition tests.

However, Copenhagen 222 and Sendai virus reacted to almost the same extent with homologous and heterologous antisera in complement-fixation tests, although they cross-reacted only slightly in haemagglutination-inhibition tests. The viruses thus appear to share a common antigen without being identical. Preliminary studies indicate that Copenhagen 222 is closely related to Haemadsorption virus type 2 [which with Sendai virus is now included in the sub-group parainfluenza 1]. The author remarks that because the virus only causes small cytopathic changes in tissue culture its presence may be overlooked unless haemagglutination tests are carried out routinely. *Janice Taverne*

LEIDY, Grace, SPRUNT, Katherine, REDMAN, Winifred & ALEXANDER, Hattie E. **Sensitivity of Populations of Clonal Lines of HeLa Cells to Polioviruses.** *Proc. Soc. Exper. Biol. & Med.* 1959, Oct., v. 102, No. 1, 81–5.

Many investigators have noted variations in sensitivity to poliovirus and in other characters of HeLa cells

maintained by routine large inoculum culture. The authors isolated 6 pure clones from their stock HeLa cell culture, using the technique devised by PUCK *et al.* (*J. Exper. Med.*, 1956, v. 103, 273). 2 of these 6 clones (Nos. 4 and 5) yielded populations of different morphology. Clone 4 was the least sensitive to poliovirus types 1 and 2. The sensitivity of this clone increased on passage, but subclones reverted to lesser sensitivity. Clone 5 yielded the most sensitive (to polioviruses 1 and 2) line and this character has been maintained during 15 months to 2 years propagation. Additional subcloning of the 6 cell lines in general did not reveal any heterogeneity in sensitivity to poliovirus of the clonal populations derived from the original HeLa cell culture. An exception to this was subclone 6 of clone 5 which was less sensitive and produced smaller plaques. These cells in clone 5 did not have a selective advantage since the sensitivity of clone 5 has remained unaltered for 2 years. The authors stress the value in quantitative studies of a pure stable population of cells but note that variations in sensitivity do occur from experiment to experiment due perhaps to variations in *p*H or serum quality or other cultural conditions. Plaques were more clearly defined in bicarbonate buffered medium than in Tris but numbers were not altered significantly. The number of cells in a monolayer, provided it is confluent, does not have much effect on sensitivity.

During 15 months' growth cells in clone 5 similar in morphology to the original HeLa cell culture and clones 1, 2, 3 and 6 appeared. Subclones of this morphology, however, still retained the enhanced sensitivity characteristic of the parental clone 5.

A. J. Beale

IONESCU-MIHĂIEŞTI, C., WISNER, B., SERGIESCO, Dina, ZAMFIRESCO, M., KLEIN, R. & HORODNICEANU, F. Sur certaines conditions régissant l'isolement, en cultures tissulaires, du virus polio présent dans le système nerveux central. Types antigéniques de ces souches (1949–1957). [**On Certain Conditions governing the Isolation in Tissue Cultures of the Polio Virus present in the Human Central Nervous System. The Antigenic Types of these Strains (1949–1957)**] *Arch. Roumaines Path. Expér. et Microbiol.* 1959, June, v. 18, No. 2, 161–8. [13 refs.]

The authors found that poliovirus could not always be recovered from the central nervous system [CNS] of fatal histologically proved cases of poliomyelitis. Thus, in the years 1949–57, 172 spinal cords were examined and 113 strains of poliovirus isolated. In 1957, 99 isolations (type I, 92, type II, 6, and type III, 1) were made from 146 cord samples. Some of the factors concerned in the isolation of poliovirus from CNS tissue were examined. Poliovirus was more readily isolated from samples from Bucharest than from those from remote parts of Rumania which took much longer to reach the laboratory. Hanks solution was found to be the best for suspending the tissue and monkey kidney cells more sensitive than HeLa. In 14 out of 25 positive spinal cords examined at 1/10, 1/100 and 1/1,000 dilution virus was detected in the diluted but not the concentrated suspension. This zone phenomenon it is speculated may be due to an inhibitor either specific antibody or non-specific. It might also be due to the production of incomplete poliovirus in the CNS by analogy with incomplete influenza virus which may interfere with the living virus. Neither of these suppositions were examined experimentally.

A. J. Beale

SALZMAN, N. P., LOCKART, R. Z., Jr. & SEBRING, E. D. **Alterations in HeLa Cell Metabolism resulting from Poliovirus Infection.** *Virology.* New York. 1959, Oct., v. 9, No. 2, 244–59, 1 fig. [27 refs.]

RNA, DNA and protein synthesis in the S3 clone of HeLa cells growing logarithmically was determined for normal cells and for those infected with type 1 poliovirus. Cells were infected at a multiplicity of 20–40 (virtually all the cells were infected), virus first appeared in the cells by 4 hours. The total viral yield was in the medium at 24 hours, but at 9, although the total yield was virtually complete, most of the virus was intracellular. Early in infection the size of the nucleotide pool increased. Protein RNA and DNA synthesis ceased, there being no increase in the first 6 hours. Thereafter RNA was lost into the medium followed between 9–12 hours by protein loss. The RNA loss into the medium occurred at the same time as the commencement of virus release but occurred at a faster pace. The amount of virus RNA synthesized was too small a proportion of the total synthesis to be detected by the methods employed. The finding of inhibition of RNA and DNA protein synthesis in poliovirus infected cells differs from those of ACKERMANN *et al.* [this *Bulletin*, 1959, v. 34, 954] but since the cell yield was 200–400 PFU/cell it is concluded that such synthesis is not required for virus production nor a consequence of virus infection. A fallacy involved in using the uptake of precursors into RNA bases as evidence for RNA synthesis is demonstrated. In these experiments there was no RNA synthesis in infected cells but an increase of the nucleotide pool which involved uptake of labelled cytidine-2-^{14}C (experiments show that uridine is actually the pyrimidine base precursor); this could have been used fallaciously as evidence of RNA synthesis. *A. J. Beale*

SELZER, Golda & BUTCHART, M. **Further Experiments on the Adaptation of Mahoney, Type 1, Poliomyelitis Virus to Suckling Mice.** *J. Hygiene.* 1959, Sept., v. 57, No. 3, 285–90, 3 figs.

The history of the successful adaptation of the Mahoney type 1 poliovirus to mice is detailed. After 6 to-and-fro passages of the virus through monkey kidney cells and suckling mouse brain, the 7th tissue culture passage fluid was inoculated into 100 mice intracerebrally. 1 mouse became paralysed within 24 hours. This virus was re-isolated in monkey kidney cells and then passed serially in suckling mice for 8 passages. At the first passage 95% of the mice were paralysed and at the 8th 33%. Despite this the titre of the brain suspension was unaltered during these passages. The virus from the 8th suckling mouse

passage level was passed once in monkey kidney tissue (9th TC passage) and then used to infect mice. This yielded a virus that could be passed regularly from mouse to mouse and caused paralysis within 3 days of 90–100%. In the first passages (75 have now been made) the brain suspension was concentrated 10 times before inoculation. Tests showed that the passage strain could still be neutralized by type 1 poliovirus ant'sera.

[For an earlier study by the author, see this *Bulletin*, 1958, v. 33, 85.] *A. J. Beale*

Ho, M. & Enders, J. F. **An Inhibitor of Viral Activity appearing in Infected Cell Cultures.** *Proc. Nat. Acad. Sci.* 1959, March, v. 45, No. 3, 385–9, 1 fig.

This very interesting paper describes the production of a viral inhibitory substance very similar to the interferon described by Isaacs and Lindenmann [this *Bulletin*, 1957, v. 32, 1203] in human kidney cells infected with a variant (RMC) of the MEF 1 strain of type 2 poliovirus. This virus has been adapted to grow in the chick embryo cells and it was found that although it multiplied slightly in human kidney cells mass destruction of the cultures was not seen. Similarly, low dilutions failed to cause destruction of human amnion cells but normal poliovirus cytopathic changes were noted with higher dilutions of virus, provided the amnion cultures were over 2 weeks old and rolled after infection. It has previously been reported that this virus fails to produce cytopathic effects in human amnion cells which are kept stationary [Frothingham, *ibid.*, 1959, v. 34, 833].

The virus inhibitory fluid (VIF) was prepared by infecting monolayers of human kidney cells with 10^5 TCD50 of RMC virus. 3 days later the tissue culture fluid was harvested as VIF. This material was then added to stationary human amnion cell cultures or human kidney cells which are not destroyed by RMC virus. It was found that VIF inhibited the action of RMC virus (on rolled amnion cells) and also types 1 and 3 poliovirus and other unrelated virus, *e.g.* Sindbis, vaccinia and herpes simplex viruses.

It was found that the effect was not produced by the simultaneous inoculation of challenge virus and RMC. Also RMC could be removed from the VIF without diminishing the effect. This was achieved by ultracentrifugation and by specific type 2 poliovirus antiserum.

The VIF appeared to act by diminishing viral multiplication since it had no direct effect on the inhibited viruses nor did it prevent their adsorption to susceptible cells. It was shown that type 1 poliovirus infection of human amnion cells was altered in the presence of VIF from a rapidly lethal infection to a chronic infection with little cytopathic effect. It is postulated that a similar mechanism may explain some of the stable infections of susceptible tissue culture cells *in vitro* with viruses. Similar inhibitors may be produced *in vivo* and have a bearing on the outcome of virus infections in the intact animal. The activity of VIF was not removed by dialysis, storage for 1 month at 4°C., or freezing and thawing. Activity was reduced but not abolished by heating to 56°C. for 30 minutes.

A. J. Beale

Takemoto, K. K. & Habel, K. **Sensitivity and Resistance of Type 1 Polioviruses to an Inhibitor in certain Horse Sera.** *Virology.* New York. 1959, Oct., v. 9, No. 2, 228–43, 8 figs.

An inhibitor of poliovirus found in the gamma globulin fraction of horse serum was studied. Plaques of poliovirus on cells overlaid with inhibitor containing serum were small (2–4 mm. at 6 days). Large plaque size (>10 mm.) mutants occasionally appeared under inhibitory serum and were antigenically indistinguishable from the small plaque mutants. Despite being in the gamma globulin fraction the inhibitor is not antibody since it does not neutralize virus. It does delay virus adsorption to and release from cells. The horse serum inhibitor is unrelated to the bovine serum inhibitor previously described and analysed [Takemori *et al.*, this *Bulletin*, 1958, v. 33, 713]. *A. J. Beale*

Li, C. P. **Studies on Variation in Virulence of Poliomyelitis Virus. IV. The Effect of 5-Bromouracil and Stable Cell Lines.** *J. Immunology.* 1959, Nov., v. 83, No. 5, 511–16, 4 figs.

The author has previously described the importance of cultural conditions for the neurovirulence in mice of a strain of Leon type 3 poliovirus. Virus grown on primary monkey kidney cells in medium 199 maintained mouse neurovirulence whereas using lactalbumin or bovine albumin hydrolysate to nourish the same cells neurovirulence was lost. Similarly, the strain was less virulent even in medium 199 when grown on HeLa cells. In this study it was found that mouse neurovirulence of the Leon strain LH-v K_{15} (15 primary monkey passages derived by 2 plaque purifications from the K_{11} pass) was reduced by 2 passages in several continuous cell lines in bovine or lactalbumin hydrolysate (MS (Monkey kidney) monkey heart, human amnion cells). Passage in a continuous rabbit kidney cell line (Drew) did not reduce virulence nor did passage in primary human amnion enhance it so that it cannot be said that the primary or stable character of the cells is the determining factor for mouse virulence.

Passage of the LH-v K_{11} in monkey kidney cells nourished with Hanks' BSS, 2% calf serum and 0·15 mgm.% 5-bromouracil did not lead to rapid attenuation. Direct inoculation of mice to determine the mouse paralytic dose did not reveal much difference between the 5-bromouracil passage line and controls in early passages. By contrast, at the 10th passage, whereas 50% of the control plaques yielded virulent progeny, none of the 5-bromouracil plaques were virulent. Similarly, only 4% of mice were paralysed against 44% of controls inoculated with the undiluted virus pool.

A. J. Beale

LE BOUVIER, G. L. **Poliovirus D and C Antigens: their Differentiation and Measurement by Precipitation in Agar.** *Brit. J. Exper. Path.* 1959, Oct., v. 40, No. 5, 452–63, 5 figs. (2 on pl.). [26 refs.]

Most poliovirus preparations contain 2 antigenic components called C and D. These components were originally separated by SCHWERDT and SCHAFFER [this *Bulletin*, 1957, v. 32, 906] using centrifugation in a sucrose density gradient. Most antisera prepared against poliovirus contain anti-D and anti-C detectable either by precipitation or complement fixation as well as neutralizing antibodies. C and D antigens are specific for one of the 3 types of poliovirus [*ibid.*, 1958, v. 33, 412, 1094]. Particles that are D antigenically appear as well defined virus particles in electron micrographs and contain 25–30% RNA. They include infective and non-infective particles, some of which sediment with the C fraction in sucrose density gradients. D containing preparations produce anti-D and also neutralizing antibodies in animals. Also anti-D and neutralizing antibodies are adsorbed together. By contrast C containing preparations used as antigen produce anti-C and no anti-D or neutralizing antibodies. C antigen is associated with damaged particles in electron micrographs which contain little if any RNA. 4 types of preparations containing C antigen exist: (i) The sucrose density gradient fraction C, (ii) fraction D heated (60°C. 20 mins.), (iii) minor fraction of crude concentrates (these contain D as a major component), (iv) crude concentrates, heat treated. All these C antigens appear identical.

From the foregoing the importance of separately measuring the C and D components or their antibodies in diagnostic serology and poliovaccine control can be appreciated. This can be done conveniently by using precipitation by specific antisera in agar. Using this technique Le Bouvier has confirmed many of the properties of D and C antigens. In particular the type specific nature but clear distinction between D and C is shown [although in some experiments cross adsorption of antibodies is described]. The evidence strongly suggests that heating D converts it to C. D antigen without detectable C still evoked anti-C in animals suggesting that C may be produced or uncovered in the body [or that the D antigen contained low levels of C]. No evidence for group or heterotypic antigens in type 1 concentrates was found, but some antisera prepared by inoculation of type 1 virus gave precipitating and neutralizing antibodies to the other 2 types. C or D antigen may be titrated by measuring the distance between serum and antigen cups and expressing the distance the precipitate moves from the serum cup as a proportion of this distance (measurements are made with calipers on photographs). It was found that there was a straight line relationship at a given serum dilution between this proportional distance and the antigen dilution. This method of measurement is less sensitive than complement fixation but enables D and C to be estimated in mixtures by using anti-C and -D sera.

A. J. Beale

SELZER, Golda. **A Comparison of Complement-Fixing Antigens by Types 1 and 2 Poliomyelitis-Infected Suckling Mouse Brains.** *J. Hygiene.* 1959, Sept., v. 57, No. 3, 321–5.

The author has previously described a small particle size complement-fixing antigen of type 2 poliovirus grown in suckling mouse brain. The complement-fixing titre of the virus fraction of infected mouse brain was lower than that of the soluble antigen component. In tissue culture little or no soluble antigen was formed [this *Bulletin*, 1958, v. 33, 1093]. This work is confirmed and mouse brain infected with the variant of Mahoney virus previously described [see above] similarly analysed. Soluble antigen was detected in such suckling mouse brain preparations and increased with the passage level of the Mahoney virus but the titre was always well below that of the virus fraction. No cross reactions were noted between the types 1 or 2 polioviruses or their soluble antigens in these complement-fixation tests. *A. J. Beale*

BRUNNER, K. T. & WARD, R. **Differences in Stability of Antigen-Antibody Complexes formed with Poliovirus and Acute and Convalescent Phase Human Sera.** *J. Immunology.* 1959, Oct., v. 83, No. 4, 405–10, 1 fig. [14 refs.]

The idea that the quality of antibody varies during the course of infection or immunization as well as quantity has been generally accepted in immunology [see BURNET and FENNER, this *Bulletin*, 1950, v. 25, 1081]. A series of 5 acute and convalescent sera from patients infected with poliovirus were examined. It was found that the kinetics of virus inactivation were similar with both sera provided no dilution of the serum-virus mixture was made before plating on to monkey kidney monolayers, although the convalescent sera contained at least 8 times more antibody. If the virus-serum mixtures were diluted 1/20 after 2 or 3 hours at 37°C. and held for 1 hour at 37°C. either at *p*H 7·5 or 4·0 it was found that a small but definite amount of reactivation occurred at *p*H 7·5 slightly more marked with acute sera. At *p*H 4·0 there was marked reactivation of acute phase sera. Convalescent sera also showed dissociation from virus greater in degree than found at *p*H 7·5 but much less than that for acute sera.

A. J. Beale

FRENCH, R. C., ARMSTRONG, R. E. & NAGLER, F. P. **Evaluation of Poliomyelitis Antisera by Plaque Neutralization Tests.** *Canadian J. Microbiol.* Ottawa. 1959, Oct., v. 5, No. 5, 461–9, 2 figs. [12 refs.]

A new method of titration of poliovirus neutralizing antibodies is proposed. In this method 500 plaque-forming units of virus in 0·5 ml. were mixed with 0·5 ml. of serum dilution and incubated at 37°C. for 3 hours. The virus was then diluted 1 in 4 and titrated for plaque forming units together with a control virus preparation. The dilution of serum neutralizing 50% of the virus was calculated. This could be done graphically since there is a straight-line relationship between

the log of the amount of virus neutralized and the volume of serum in the test. It was found that the accuracy of the plaque assay was within ± 25% of the mean value and that types 1 and 2 antibody titrations were within 25% and type 3 within 45% of the mean. The plaque neutralization method was found to give titres for British Standard poliomyelitis antisera 1·2 times higher than those obtained by the inhibition of the cytopathic effect with 3 hours at 37°C. as reaction time. The titres were about 4·7 times higher than the titres obtained for American reference sera in a metabolic inhibition test holding the virus-serum mixtures 1 hour at room temperature.

[This technique may prove of great value since present techniques of serum neutralizing antibody determinations frequently vary 2–4-fold even when expressed in terms of a ratio to a reference serum, see PERKINS and EVANS this *Bulletin*, 1939, v. 34, 1057.]

A. J. Beale

FRENCH, R. C., ARMSTRONG, R. E. & YAROSH, W. **Improvements in the Tissue Culture Safety Testing of Salk Vaccines.** *Canadian J. Microbiol.* Ottawa. 1959, Oct., v. 5, No. 5, 453–9, 2 figs. [17 refs.]

This valuable contribution describes experiments aimed to improve the safety test for killed poliovaccine carried out in the Canadian control laboratories in Ottawa. The rate of poliovirus (the experiments were done with the MEF1 strain of type 2) inactivation at 37°C. was linear at 0·0005 per minute, *i.e.* 9% inactivated in 4 hours and 80% in 3 days. Adsorption of poliovirus to monkey kidney monolayers overlaid with 100 ml. of medium in Roux bottles was also approximately linear, 14% being adsorbed in 4 hours and by extrapolation 93% in 3 days. From these results it was concluded that (a) under the condition involved in the manufacture of poliovaccine 99% of the virus would be inactivated in the absence of formaldehyde and (b) the fluid in safety test cultures could be changed after 4 days without impairing the safety test. From the adsorption constant it was calculated that if the volume/cell ratio was reduced from 0·67 ml. to 0·17 ml. per sq. cm. of cells then 97% of the virus would be adsorbed in 24 hours instead of 58%. Since heat inactivation is proceeding simultaneously this should lead to a greater sensitivity. This expectation was realized in tests with virus containing 1 plaque-forming unit per ml. In order to be able to utilize smaller volumes of inoculum (to achieve low volume/cell ratios) the samples for safety test were concentrated on the Spinco ultracentrifuge at 80,000 g for 3 hours in 0·06% gelatin.

800 ml. of vaccine to which live virus had been added in sufficient quantity to give 1 plaque-forming unit in 40 ml. was tested in 2 ways. (1) 400 ml. was inoculated in 40 ml. amounts in Roux bottles containing 80 ml. of medium. The cells were completely changed after 7 days and 14 days, 10% of the fluid being subcultured into smaller flasks. The test was terminated at 3 weeks. (2) The remaining 400 ml. was concentrated 4-fold by ultracentrifugation and 10 ml. inoculated into a T-60 flask (0·17 ml. per sq. cm.). At 7 days the cultures were changed into 20 ml. and 10% of the spent fluid subcultured. The test was also terminated at 3 weeks. Test B yielded 96/120 isolations in a series of comparisons and test A 60/120—differences that were highly significant.

[In most of these experiments untreated poliovirus at low dilution was used to simulate conditions in a safety test for residual poliovirus. In fact there is evidence that virus partially inactivated by formaldehyde behaves differently from native virus in that it multiplies more slowly in culture mainly but not entirely because of slower adsorption (BEARDMORE *et al*, this *Bulletin*, 1958, v. 33, 714).]

A. J. Beale

MELNICK, J. L., BENYESH-MELNICK, Matilda & BRENNAN, J. C. **Studies on Live Poliovirus Vaccine. Its Neurotropic Activity in Monkeys and its Increased Neurovirulence after Multiplication in Vaccinated Children.** *J. Amer. Med. Ass.* 1959, Oct. 31, v. 171, No. 9, 1165–72. [19 refs.]

An independent assessment of some of the problems associated with the use of attenuated poliovaccine is presented. The neurovirulence of Sabin's and Lederle's strains was assessed by intracerebral (1 ml. into the thalamus) and intraspinal (0·1 ml. into the lumbar cord) inoculations of cynomolgus monkeys. It was found that the strains were considerably more neurovirulent in these tests than in the tests performed by their sponsors. The recommended dose of all 3 types of Lederle attenuated vaccine contained over 1,000 MPD50 (dose causing specific lesions in 50% of the animals given an intraspinal dose). Even by the intracerebral route the type 1 and type 2 strains caused specific lesions in monkeys in the recommended dosage for human beings. Sabin's strains were less neurovirulent, causing only very rare lesions by the intracerebral route. These strains, however, in the recommended dose for human prophylaxis contained for type 1 1,000 MPD50, type 2 more than 25 MPD50, and type 3 100 MPD50 by the intraspinal route. [This is based on a human dose of about 100,000 TCD50 but recently 100 times this dose has been used, see HORSTMANN *et al.*, this *Bulletin*, 1959, v. 34, 1085.] The technique of performing the intraspinal inoculation differs from that advocated by Sabin and is criticized by him as being too traumatic. 0·1 ml. is inoculated intraspinally into the first space below the last rib. An American 27-gauge needle is used and the needle is positioned (which may mean moving it) so that continuous leg muscle contraction or fibrillation occurs during the injection. In this way it is ensured that the inoculum goes into the correct part of the cord. Control fluids, either a non-paralytogenic ECHO virus or polioviruses diluted beyond the end point, failed to produce lesions that could be confused with those specific for poliomyelitis.

36 out of 81 strains of poliovirus isolated from children in Mexico given Sabin's attentuated strains orally, or their contacts, have been examined for genetic stability. In selecting strains for neurovirulence studies those that had reverted from the d⁻ or t⁻ character of the vaccine strains were chosen. [d+ is the ability to grow in the presence of low levels of bicarbonate and t+ the ability to multiply at 40°C.] 15 were unchanged d⁻ t⁻, 16 reverted

to d+ t- and 5 to d+ t+. The d- t- were of unaltered neurovirulence. The d+ t- were slightly more virulent, 10 TCD50 contained 1 MPD50 (dose causing clinical paralysis by the intraspinal route) as against 10,000 TCD50 for the d- t-. d+ t+ were more virulent still, producing paralysis by the intracerebral route in monkeys direct from the rectal swabs.

The adequacy of many tests for the safety of attenuated strains of poliovirus is questioned. It is estimated from the results of the Cutter incident [EKLUND *et al.*, *ibid.*, 1959, v. 34, 25] and of observations on a severe type 1 epidemic [MELNICK & LEDINKO, *ibid.*, 1954, v. 29, 137, 207, 1953] that only 1 out of 500 to 1,000 children infected with a virulent strain succumb with paralysis. It is concluded, therefore, that very large-scale tests in susceptible populations are required before safety can be established. It is known that many factors besides neutralizing antibodies determine the outcome of poliovirus infection. Since the attenuated viruses can spread and are genetically unstable, and so little is known about the factors that influence the outcome of the disease in an individual or a population, it is thought that more knowledge is required before the strains can be advocated confidently. One of the factors affecting the spread of poliovirus in the community and the effectiveness of attenuated poliovaccine is the presence of other viruses in the alimentary tract. This is illustrated by studies made in children from Mexico City. 22 out of 28 children not excreting a virus before vaccination acquired antibodies whereas 33 out of 47 who were excreting virus failed to acquire homotypic antibody after the administration of attenuated poliovirus.

Other formidable problems of attenuated poliovaccine manufacture are discussed, but no new evidence is brought forward. The testing of different batches for consistency is one problem. The fact that the virus will be prepared in monkey kidney tissue which potentially may contain one of a large number of simian viruses is another. The pathogenic properties of one of these is known (virus B is a lethal human pathogen though the lethal dose is unknown) but there is no information about the others.

The authors are careful to point out that their results do not show that attenuated polioviruses are unsafe. They do, however, urge caution. If caution was required in the introduction of inactivated vaccine [the Cutter incident was the price of haste] it is even more necessary for a living vaccine capable of spreading uncontrollably in the community.

[One of the disturbing features of attenuated polioviruses is the lack of agreement between authors. First, the sponsors of the vaccine tend to find them less virulent than independent workers. Sabin ascribes these discrepancies to variations in technique, which is probably correct but not very reassuring. Second, the antibody responses and duration of immunity vary, for example the results of DICK and DANE [*ibid.*, 1959, v. 34, 200] with KOPROWSKI's strains and CLARKE *et al.* [*ibid.*, 201] with Sabin's type 3 are less good than those recorded by the sponsors. Third, the presence of simian viruses in attenuated poliovaccine can be the subject of acrimonious dispute rather than ascertainable fact [KOPROWSKI, *ibid.*, 955]. The need for independent confirmation of the work on attenuated polioviruses is therefore urgent. This paper should be read in full as just such an independent enquiry.] *A. J. Beale*

LI, C. P. **Experimental Variation in Mouse Virulence of Echo 9 Virus.** *Proc. Soc. Exper. Biol. & Med.* 1959, Oct., v. 102, No. 1, 223–4.

The passage of ECHO 9 virus Bourn strain (LR $451K_8$ P_1 K_1) [this *Bulletin*, 1957, v. 32, 829] in MS cells (stable monkey kidney cells) in Eagle's medium with 2% serum resulted in a lowering of its virulence for mice compared with control cultures passaged in primary monkey kidney cells in parallel. The titre of the virus grown in MS cells was less than in monkey kidney, but this titre difference was not responsible for the difference in virulence. *A. J. Beale*

BUCKLAND, F. E., BYNOE, M. L., PHILIPSON, L. & TYRRELL, D. A. J. **Experimental Infection of Human Volunteers with the U-Virus—a Strain of ECHO Virus Type 11.** *J. Hygiene* 1959, Sept., v. 57, No. 3, 274–84, 5 figs. [15 refs.]

Philipson has previously described a virus which he isolated from children with croup [this *Bulletin*, 1958, v. 33, 995, 1188]. This virus has now been found to have the characters of the ECHO group being about 27 mμ in diameter and resistant to ether. Antigenically it appears to be a strain of ECHO 11 virus. The virus was fed to human volunteers in 2 forms. (a) Virus grown on human embryonic lung cells in bovine amniotic fluid [this was the same material used by Philipson, *ibid.*, 1188]. (b) Virus given 11 serial passages in monkey kidney cells in medium 199. 100,000 TCD50 of virus was instilled into the noses of volunteers. All the volunteers gave serological evidence of infection with a rise in neutralizing and haemagglutinating antibodies (1 volunteer showed only a 3-fold rise) but the virus was more readily isolated from those who received virus grown on human embryo lung tissue. Similarly, 8 of the 9 volunteers infected with the virus grown on human embryo lung developed a mild but definite illness with malaise or gastro-internal symptoms, whereas only 3 out of 8 developed any symptoms (2 transient fever and 1 sore throat) after infection with virus passed 11 times in monkey kidney cells. Pooled sera from various age-groups collected in Sheffield in 1955 by the method described by DAVENPORT *et al.* [*ibid.*, 1956, v. 31, 6] contained haemagglutination inhibiting antibodies. 39 out of 5 sera from persons aged 18–45 collected in 1957–58 contained antibodies in titres varying from 1/4 to 1/96. It is concluded that U virus is a strain of ECHO 11 and that there is evidence of widespread infection of the population with it. Passage in monkey kidney cells appears to attenuate the virus and it is suggested this means that a living vaccine could be readily prepared if the need arose.

[In view of the fact that monkey kidney cells are known to contain many viral agents [HULL *et al.*, *ibid.* 873], details of the measures taken to exclude these agents would be helpful.] *A. J. Beale*

KARSTAD, L., SPALATIN, J. & HANSON, R. P. **Experimental Infections of Wild Birds with the Viruses of Eastern Equine Encephalitis, Newcastle Disease and Vesicular Stomatitis.** *J. Infect. Dis.* 1959, Sept.– Oct., v. 105, No. 2, 188–95, 2 figs. [13 refs.]

Crows (*Corvus brachyrhynchos*) and doves (*Zenaidura macroura*) were infected orally, subcutaneously, intracardially and intracerebrally with vesicular stomatitis virus (Wisconsin strain), but no clinical or histological changes were detected and no antibody formation resulted.

Fatal infections in 3–11 days resulted from subcutaneous and intracerebral inoculation of crows with Newcastle disease virus (Texas GB strain), while oral and intracardiac infection caused only antibody formation. Viraemia was demonstrated in 3 birds 48–72 hours after infection. Histology revealed congestion and slight meningitis in the brains of fatally infected birds.

Crows infected subcutaneously, orally and intracerebrally with eastern equine encephalomyelitis virus (chick-embryo passage) showed no symptoms but had histological changes in their brains and antibody was formed. Oral infection was by feeding the birds on infected chick-embryos. Virus could not be isolated from the tissues of adult crows 14 days after infection, but could be isolated from brain, lung, liver and spleen of nestling crows infected 5 days before. The histological changes in the brains were meningitis, glial cell proliferation, perivascular leucocytic infiltration and neuronal degeneration.

Doves were infected successfully with eastern equine virus intracerebrally, subcutaneously and intracardially, but not orally. The results were similar to those in the crows. An owl (*Bubo virginianus*) and 2 hawks (*Buteo jamaicensis* and *B. lagopus*) were infected intracardially. The owl and 1 of the hawks showed strong antibody responses but the *B. lagopus* had a low response. Virus was isolated from the brain of the owl 11 days after infection in the presence of circulating antibody. All 3 birds had histological changes in their brains. *C. E. Gordon Smith*

SOKOL, F., LIBIKOVÁ, H. & ZEMLA, J. **Infectious Ribonucleic Acid from Mouse Brains infected with Tick-Borne Encephalitis Virus.** [Correspondence.] *Nature.* 1959, Nov. 14, v. 184, Suppl. No. 20, 1581.

By phenol extraction of mouse brains infected with the Hypr strain of tick-borne encephalitis virus, infective ribonucleic acid (RNA) has been prepared. Its infectivity was 10^{-3} to 10^{-5} of that of the original material, and it was destroyed by heating at 37°C. with ribonuclease while that of the original material was little affected. The infectivity of the RNA was unaffected by heating only. Neutralization with specific gamma globulin reduced the titre of the original material 500–12,600-fold, but that of the RNA only 5–6-fold. Ethanol precipitation destroyed the infectivity of the original material, but not that of the RNA. The RNA caused typical central nervous system disease on inoculation into mice and the resulting virus was identified by neutralization tests. *C. E. Gordon Smith*

LIU, Yuan-Yuan, CHANG, Han-King, CHANG, Chi-Shiang & KUAN, Hsüeh-Chin. [**Studies on the Purification of the Japanese B Encephalitis Virus. I. Influence of pH on the Infectivity of the Virus—the pH Elution Curves and the Isoelectric Point of the Virus.**] *Acta Microbiologica Sinica.* 1959, Nov., v. 7, No. 3, 164–6, 2 charts. [In Chinese.]

The English summary appended to the paper is as follows:—

"The pH elution curves and isoelectric point of the Japanese B encephalitis virus (Peking strain) were studied by determining the infective titres of the virus in the supernate of the 10% infective mouse brain suspension at various pH conditions and in the supernate obtained after resuspending the residue in buffer saline. It was observed that when the infective mouse brain was suspended in buffer saline above pH 7.0, the virus titre of the supernate was frequently higher than that of the control and of the resuspended suspension. However, it began to drop quickly below pH 6.0. Lowest infective titre was reached below pH 4.0. Under the latter condition, higher titres were observed in the resuspended suspensions than in the original supernates although they were far beneath the level of the control titre. No sharp drop in titre either in the original suspension or in the eluate was observed above pH 4.0. It was therefore believed that the isoelectric point of the virus may lay somewhere between pH 3.0–4.0, probably at pH 3.5 The optimal pH for maintaining the infectivity of this virus is found to be at 7.8–9.6 in phosphate buffer saline."

LIU, Yuan-Yuan, CHANG, Han-King, CHANG, Lee-Pee, CHANG, Chi-Shiang & KUAN, Hsüeh-Chin. [**Studies on the Purification of the Japanese B Encephalitis Virus. II. Purification of the Virus from Mouse Brain by means of the Combined Action of certain Salts and Adsorbing Substances.**] *Acta Microbiologica Sinica.* 1959, Nov., v. 7, No. 3, 167–74. [22 refs.] [In Chinese.]

The English summary appended to the paper is as follows:—

"The application of the combined action of certain salts and adsorbing substances for the purification of Japanese B encephalitis virus ($Peking_1$ strain) from mouse brain suspension has been studied. It was found that when the virus suspension was about half saturated with ammonium sulfate (3 *M.*, at pH 7.8), the addition of 10% kaolin brought about an almost complete precipitation of the virus. The precipitated virus could be eluted with *M*/200 phosphate saline at pH 8.6. In most cases, no drop in the infective titre was observed in the eluates and the total nitrogen content was only about 5% of that of the original 10% mouse brain suspension. Addition of 0·5% protamine sulfate to the purified material precipitated most of the remaining mouse brain protein without largely influencing the virus titre. Dialysis against running tap water under 15°C overnight to remove the ammonium ion occasionally led to a slight decrease in the virus titre."

LIU, Yuan-Yuan & HOU, Yun-Te. [**Influence of the Multiplication of the Japanese B Type Encephalitis Virus on the Activity of Xanthine Oxidase of the Host Tissues.**] *Acta Microbiologica Sinica.* 1959, Nov., v. 7, No. 3, 175–82, 1 chart. [11 refs.] [In Chinese.]

The English summary appended to the paper is as follows:—

"The influence of the multiplication of three strains of Japanese B type encephalitis virus, i.e. $Peking_1$ strain, Nakayama strain and M_{47} strain on the activity of xanthine oxidase in extracts of the infected mouse brain and liver tissues were studied. It was observed that the enzyme activity was increased in all cases of infection. There was a significant difference between the results obtained from the brain extracts of Nakayama and M_{47} strains, but no significant difference being observed between those of $Peking_1$ and Nakayama strains or $Peking_1$ and M_{47} strains.

"A relationship between the levels of the increment of enzyme activity in all cases and the latent period of the disease was observed. When the quantity of virus injected was smaller, the infected animals would live longer and the enzyme activity of the infected tissue extracts would reach a higher level than when larger quantity of virus was injected. The possibility of grouping viruses according to their xanthine oxidase inducing capacities was discussed in the paper.

"The experiment, however, proves that the increase of enzyme activity is the result of virus multiplication and not a nonspecific reaction. When a smaller quantity of virus (about $10^{4.5}$ LD50 dose) was injected into immunized mice, the virus could not be detected either in the brain or the liver of the infected animals. No multiplication of the virus could be observed after infection, in the same time, no increase of xanthine oxidase activity was detected."

LI, Ho-Ming & YU, Yung-Shing. [**Growth of the Japanese B Encephalitis Virus in Simple Tissue Culture.**] *Acta Microbiologica Sinica.* 1959, Nov., v. 7, No. 3, 183–8, 2 charts. [In Chinese.]

The English summary appended to the paper is as follows:—

"By the use of minced chicken embryonic tissues free from the brain tissue and placed in Tyrode solution containing 10% embryonic extract and 20% rabbit serum, it has been possible to obtain a virus titre between 10^{-5} and 10^{-6} when the flasks were incubated between 34 and 36°C. It was found that the amount of fluid in the flask should be so adjusted that it was not more than 1 mm in depth, and the total volume of the tissue should not be more than one thirtieth of the fluid.

"In such culture, the maximum titre of the virus was reached by the fourth day; but after that, it maintained this titre up to the 16th day. At first, there was more virus in the fluid than in the tissue suspensions but later on the reverse was true.

"It was found that this simple culture method is more sensitive than the intracerebral injection of the white mice in detection of the virus, and the vaccine prepared from such cultures also possesses high protective action against the white mice."

SMITHBURN, K. C., PATERSON, H. E., HEYMANN, C. S. & WINTER, P. A. D. **An Agent related to Uganda S Virus from Man and Mosquitoes in South Africa.** *South African Med. J.* 1959, Nov. 14, v. 33, No. 46, 959–62.

Blood, from an African aged 9 years in Tongaland with a mild fever, was inoculated into infant and adult mice. The infant mice sickened on the 5th day and brain passage resulted in the isolation of a filterable agent (H 336). Re-isolation was successful. Although the original material was not pathogenic for adult mice, passage material was. A specimen of serum from the patient 1 month later neutralized the virus. No symptoms were felt by the patient. From a pool of 34 *Culex* (*Neoculex*) *rubinotus* trapped near Johannesburg a similar virus (AR 1115) was isolated.

The virus causes diffuse vesicular and pyknotic degeneration of nerve cells, with moderate or absent perivascular infiltration. In mice intracerebral and intraperitoneal titres are about the same. The virus is not pathogenic for guineapigs. A non-immune monkey (*Cercopithecus* sp.) inoculated subcutaneously did not show symptoms and circulating virus was not detected.

The virus was compared in neutralization tests with a wide variety of arborviruses and relationships demonstrated in low degree only with Wesselsborn, Zika, dengue-1 and yellow fever viruses. Cross-neutralization and cross-vaccination tests showed that it was closely related to Uganda S virus, but not identical with it. 280 human survey sera were tested for neutralizing antibody to H 336 and Uganda S viruses: results agreed in 76% and disagreed in 10%, the remainder being inconclusive. On the other hand, when 120 sera were tested against H 336 and AR 1115, results only disagreed in 1 serum.

The female progeny of wild caught *Culex* (*Culex*) *univittatus* were fed on a mouse brain suspension of H 336. 14 mosquitoes fed. They were induced with difficulty to bite suckling mice, 6, 7, 8, 9, 13 and 14 days after feeding. Transmission was achieved on each occasion.

Antibodies to H 336 are common in the Tonga lowlands and in Mozambique. *C. E. Gordon Smith*

ISAACS, A. & WESTWOOD, Marguerite A. **Duration of Protective Action of Interferon against Infection with West Nile Virus.** [Correspondence.] *Nature.* 1959, Oct. 17, v. 184, Suppl. No. 16, 1232–3, 1 fig.

Tubes of trypsinized chick embryo cells maintained in lactalbumin hydrolysate and buffered salt solution were inoculated with West Nile virus (Egypt 101). The medium was changed at intervals and titrated for haemagglutinin. Peak titres were found after 4 days incubation by which time cytopathic changes were extensive.

Interferon was prepared by incubating irradiated influenza virus with chorio-allantoic membranes. When

interferon was included in the initial medium the cells infected with West Nile virus remained healthy for 11 days and haemagglutinin was not produced. Interferon did not inhibit haemagglutination in controls. In another experiment only 2 tubes on a single day (7th) produced haemagglutinin.

In similar experiments where the maintenance medium had added 5% calf serum and 1·5% chick embryo extract, similar results were obtained in the first 2–3 days, but the cells then rapidly degenerated.

Cells treated with interferon and infected with West Nile virus were therefore maintained without serum or embryo extract for 3, 4 or 5 days then serum and embryo extract added. Metabolism at once increased, but resistance to viral multiplication partially broke down.

From these experiments it appears that when cell division occurs, the interferon, which is unable to replicate, is diluted until the concentration within the cells is too low to inhibit viral multiplication. The inhibition could be largely maintained if further interferon was added each time serum and embryo extract was added. *C. E. Gordon Smith*

LAVILLAUREIX, J. & VENDRELY, Colette. Étude cytophotométrique du contenu en acide désoxyribonucléique des noyaux de cellules KB au cours de leur infection par le virus West Nile. [**Cytophotometric Study of the DNA Content of the Nuclei of KB Cells During Infection with West Nile Virus**] *C.R. Soc. Biol.* 1959, v. 153, No. 7, 1239–41, 2 figs.

IWASA, S. **Multiplication of Rift Valley Fever Virus in Human Liver Cell Culture with special reference to Production of Complement Fixing Antigen.** *Japanese J. Exper. Med.* 1959, Aug., v. 29, No. 4, 323–34, 2 text figs. & 4 figs. on pl. [23 refs.]

A pantropic strain of Rift Valley fever virus was used. The serum of infected mice containing 10^{10} 10LD50 of virus per ml. was used to infect tubes of Chang's human liver cells. Cytopathic changes were seen after 24–48 hours. 12 serial passages were made with titres varying from $10^{5·8}$ to $10^{8·3}$ plaque-forming units per ml. Plaque titrations were made in monolayers of liver cells. Plaques were visible by the 4th day and increased in size until the 5th day. Maximum absorption of the inoculum to the monolayer required 90 minutes incubation before the agar overlay was applied. Plaque count titres were somewhat lower than corresponding titres obtained by mouse inoculation. The plaque count was found to be directly proportional to the amount of the inoculum plated. Freezing and thawing of the cultures increased the virus yield. Virus in the cultures began to increase about 6 hours after infection then rapidly increased for 2½ hours. Complement-fixing antigen could be detected about the 5th hour and was at maximum titre (1/8) from about the 7th to the 9th hour. The appearance of antigen in the cultures was also studied with fluorescent antibody. The antigen was first detected in the cytoplasm 5 hours after infection and increased until the whole cytoplasm was filled with fluorescent material.

C. E. Gordon Smith

HOLDEN, P. & HESS, A. D. **Cache Valley Virus, a Previously Undescribed Mosquito-Borne Agent.** *Science.* 1959, Oct. 30, v. 130, 1187–8.

The virus was isolated from a pool of 50 wild-caught *Culiseta inornata* from Cache Valley, northern Utah, by inoculation into young adult mice. The mice were moribund in 8–10 days and died by 13 days. Reisolation was successful. The virus was filterable. In young adult mice 11 passages failed to give high titres, but 3 passages in suckling mice yielded virus with a high titre ($10^{-7·7}$) in young adults. The virus was not pathogenic for adult mice by subcutaneous or intraperitoneal inoculation, nor intramuscularly in rabbits, nor subcutaneously in ½-day-old chicks. It was cytopathic for hamster kidney cells. 4 horses from Cache Valley were found to have neutralizing indices of 50 to over 500. 3 other horses and 5 men from the same area did not have antibody.

Cache Valley virus has been shown to belong to the Bunyamwera group of arborviruses (which also includes Bunyamwera, Wyeomyia and Kairi (Tr 8900) viruses). Similar viruses have been isolated in Montana and Brazil. *C. E. Gordon Smith*

HORVÁTH, S. **A Quantitative Semi-Micro Tissue Culture Method and its Use in Microbiology. II. Use of the Method in titrating the Virus of Aujeszky's Disease.** *Acta Microbiologica.* Budapest. 1959, v. 6, No. 1, 5–16, 7 figs. [15 refs.] **III. Use of the Method in studying the Action of Diverse Chemical Substances and Gas Gangrene Bacillus Toxins on Chicken-Heart Fibroblasts.** *Ibid.*, 17–21, 3 figs. [10 refs.]

The semi-micro method of tissue culture previously developed by the author (*Acta Microbiologica*, 1958, v. 5, 415) has been applied to the titration of pseudorabies virus. Maitland-type cultures of chick heart were prepared by placing 3 pieces of tissue in a plasma coagulum inside a 2 cc. ampoule, followed by culture fluid containing 0·5% egg white. The cultures were rotated for 2 days and then infected with serial dilutions of the virus; multiplication of the virus produced cell degeneration. The culture method could also be used for quantitative measurement of the cytotoxic effect of crystal violet, brilliant green, mercuric chloride and the toxins of *Clostridium oedematiens, Cl. welchii* and *Cl. septicum.* *D. J. Bauer*

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NOSSAL, G. J. V. **Antibody Production by Single Cells. III. The Histology of Antibody Production.** *Brit. J. Exper. Path.* 1959, Aug., v. 40, No. 4, 301–11, 10 figs. (6 on pl.).

Popliteal lymph nodes from rats, previously injected in the foot-pad with Salmonella Adelaide flagellar antigen, were excised, and lymph node cell suspensions made. Single cells were transferred by micro-manipulation to tiny drops of suspending medium separated from each other by paraffin oil in a special chamber. These methods have been described by the author in previous reports [this *Bulletin*, 1959, v. 34, 403, 958].

After incubation of the oil chamber for 4 hours at 37°C. motile Salm. Adelaide cells were introduced into the drops in order to detect by immobilization of the organisms any antibody which might have been released into the drop by the lymph node cell. The histological type of cell in each drop was later determined by introducing a mixture of acetic acid, orcein, and light green into the drop to fix and stain the cell which was then examined microscopically.

Cells could be identified by this staining procedure as lymphocytes, macrophages, blast cells, plasmablasts, and plasma cells. Antibody was produced by 8 of 43 plasmablasts examined, by 83 of 187 plasma cells, by 1 each of 66 blast cells and 306 lymphocytes, and by none of 23 macrophages and 2 polymorphs. Antibody-producing plasma cells were not found in appreciable numbers until 7 days after a primary antigenic stimulus and 4 days after a secondary stimulus. The author considers that the finding of the blast cell and the lymphocyte-producing antibody could possibly be explained by technical difficulties in the technique of antibody detection or in cell identification, and concludes that the plasma cell series are the main if not the only antibody-forming cells of the body.

I. B. R. Duncan

WEIGLE, W. O. & DIXON, F. J. **The Antibody Response of Lymph Node Cells transferred to Tolerant Recipients.** *J. Immunology.* 1959, June, v. 82, No. 6, 516–19, 1 fig. [16 refs.]

This work sought a reason for the failure of lymphoid cells transfused into an irradiated rabbit to form antibody in response to a primary antigenic stimulus. Donor rabbits were killed and suspensions of lymphoid cells prepared from their mesenteric and popliteal lymph nodes. Suspensions were made from the nodes of normal rabbits and of rabbits immunized with bovine gamma globulin and bovine serum albumin. The antigens were labelled with radio-active iodine (^{131}I).

Immune tolerance to these proteins was produced in 17 rabbits by injecting them with the antigens within the first 5 days of life. Another 14 rabbits were irradiated with 400 r of X-rays. Antibody responses after the transfusion of lymphoid cells to immune or irradiated rabbits were detected by injecting the animals with ^{131}I labelled antigen immediately after the lymphoid cells and determining the speed of elimination of the antigen from the animals' sera. Rapid elimination indicated production of antibody by the transfused cells since the recipient rabbits were themselves incapable of forming antibody.

Rabbits showing immune tolerance were injected with already sensitized lymphoid cells and with antigen. Rapid elimination of antigen demonstrated a rapid secondary antibody production. Irradiated rabbits and rabbits showing tolerance were transfused with normal lymphoid cells and injected with antigen. Of 15 tolerant animals 8 showed rapid immune elimination of antigen, and all of 14 irradiated rabbits failed to do so.

The authors suggest that since transfused cells can form antibody after primary antigenic stimulation in the tolerant but not in the irradiated animal, radiation must damage the antibody-forming cells not only directly but also in an indirect way during the period after radiation has ceased. *I. B. R. Duncan*

GELZER, J. & SUTER, E. **The Effect of Antibody on Intracellular Parasitism of *Salmonella typhi-murium* in Mononuclear Phagocytes *in vitro*. Prolonged Survival of Infected Monocytes in Presence of Antibody.** *J. Exper. Med.* 1959 Nov. 1, v. 110, No. 5, 715–30, 1 text fig. & 4 figs. on pl. [41 refs.]

Rabbit mononuclear phagocytes and suspensions of *Salmonella typhi-murium* were incubated together either in the presence, or absence, of homologous antiserum. After a period of some 40 minutes or so, during which time phagocytosis had occurred, the infected cells were removed from the mixture and washed to remove the extracellular bacilli and any free antibody. The cells were then reincubated in a medium which did not contain antibody and the interaction between the mononuclear phagocytes and the bacteria was studied.

Whether antibody was present during phagocytosis or not the bacteria appeared to grow within the cells, but when antibody was absent the cells, within a few hours, began to degenerate and there was seen to be obvious extracellular multiplication of the bacteria. When, however, antibody had been present the mononuclear phagocytes could be seen clearly defined and packed with bacteria; there was little or no extracellular growth of bacteria.

The anti-salmonella serum did not promote phagocytosis and, even with the addition of fresh complement, did not appear to be bactericidal. By the use of fluorescent antibody technique it was possible to demonstrate the presence of antibody within the infected mononuclear phagocytes where it seemed to fit round the bacterial clump as an overcoat. It is suggested that the antibody was carried in by the bacteria as they were engulfed by these cells.

It was found that the active factor in the serum was gamma globulin and that it reacted solely with the somatic antigens of *Salm. typhi-murium*; the anti-flagella fractions played no part. The role of the antibody appeared to be a protective one. Without

interfering with the multiplication of the bacteria, at any rate for some considerable time, the antibody seemed to protect the cell against the early degeneration that was seen in the cells in which phagocytosis occurred in the absence of antibody.

[When bacteria are artificially cultured within cells which normally do not phagocytose organisms it is equally important to prevent extracellular multiplication of the organism under culture in order to preserve the cells. In the case of *Brucella* culture in HeLa tissue-culture cell-system recently described by SHEPARD [see this *Bulletin*, 1960, v. 35, 171] once phagocytosis had occurred the infected cells were removed from their original medium and transferred to a special serum medium containing streptomycin which did not affect the intracellular growth but controlled extracellular multiplication.] *H. J. Bensted*

LANDY, M., TRAPANI, R. J. & SHEAR, M. J. **Inactivation of Endotoxin by a Humoral Component. IV. Alteration in the Immunological Properties of Typhoid Endotoxin.** *J. Exper. Med.* 1959, Nov. 1, v. 110, No. 5, 731–50, 1 fig. [26 refs.]

It has generally been accepted that the antigenicity of the typhoid endotoxin is closely associated with its toxicity, so close has been the bond that it has not been considered possible to separate the two. Mammalian blood contains a component capable of detoxifying the endotoxin. This endotoxin-detoxifying component (EDC) so alters these bacterial products (endotoxins) that the host no longer responds characteristically—that is in the way of a specific immunological response—and the paper is concerned with the study of this effect.

Employing a Boivin type antigen prepared from *Salmonella typhi* the authors found that the average antibody response in rabbits increased as the dose of endotoxin was increased, but incubation with human plasma greatly decreased the antigenicity of the endotoxin. The addition of Ca ions, however, suppressed this action.

Hydrolysis of the typhoid endotoxin with acetic acid yields a well characterized haptenic polysaccharide of relatively low (20,000) molecular weight which is no longer antigenic for the rabbit, but which is still capable of specifically combining with antibody and precipitating it. There are suggestive similarities between the properties of EDC-altered typhoid endotoxin and the haptene, but they may not be identical. The molecular weight of a potent typhoid endotoxin is in the region of a million or more and it is possible that EDC may have split the macromolecule into smaller polysaccharide units.

It is considered that the tight bond between antigenicity and toxicity is due more to alterations in the responses of the host rather than to something belonging to the endotoxin itself. Suitable modification of the host permitted the toxic type of response to be elicited without an antigenic response and conversely there could be antigenicity without toxicity. Thus: (a) Prior treatment with thorotrast increased the sensitivity to the effect of endotoxin but did not increase antigenicity. (b) Substitution of the toxicity of a second endotoxin for the one removed from typhoid endotoxin by EDC did not restore the antigenic potency. (c) The toxicity furnished by a second endotoxin to increase the toxic reactions in rabbits receiving a minimal immunizing dose of typhoid endotoxin did not enhance the antibody response to the latter. (d) In rabbits made tolerant to the (toxic) effect of endotoxin elicitation of the usual antibody response to typhoid antigen was not prevented.

[For earlier parts, see this *Bulletin*, 1959, v. 34, 297.] *H. J. Bensted*

ROSEN, F. R. & LANDY, M. **Inactivation of Endotoxin by a Humoral Component. V. EDC Activity during Gramnegative Infection and Shock.** *Ztschr. f. Immunitätsf. u. Exper. Therap.* 1959, Oct.–Nov., v. 118, Nos. 4/5, 262–8. [10 refs.]

"The activity of an endotoxin detoxifying component in blood (EDC) was examined during the course of a variety of injuries inflicted on experimental animals as well as in naturally occurring pathological conditions in man. No significant alteration in EDC activity was discernible in experimental animals subjected to irreversible hemorrhagic and burn shock, radiation injury or infection with Gram-negative pathogens. However in five human subjects suffering from Gram-negative infection associated with other pathological conditions, EDC activity was either absent or greatly depressed. In some 30 patients with disease not directly related to infection with Gram-negative organisms EDC activity was within normal limits."

DIXON, F. J., KUHNS, W., WEIGLE, W. O. & TAYLOR, P. **The Lack of Absorption of Ingested Bovine Antibody in Humans.** *J. Immunology.* 1959, Oct., v. 83, No. 4, 437–41.

This describes a failure to demonstrate an increase in circulating diphtheria antitoxin in 3 groups of human subjects who ingested bovine milk and serum rich in diphtheria antitoxin.

10 infants 1 day old, born at term, 3 infants 2–3 months old and 11 Schick-positive medical students, were given by mouth bovine serum and milk, rich in diphtheria antitoxin, for 3 days in amounts proportional to body weight.

By haemagglutination and rabbit skin neutralization tests, the amount of diphtheria antitoxin in the serum was titrated before the feeds, and 24 hours after completing the feeds. The second serum samples of 5 neonates and 5 students were tested for bovine gamma globulin by Ouchterlony's method. 4 students were Schick tested before and after the feeds.

The authors found no significant alteration in the Schick reactions of those tested and no significant rise in antitoxin levels by serological testing except in one case, where the haemagglutination titre was raised. Bovine gamma globulin was not detected in any subject.

The subject showing the rise in haemagglutination titre had no demonstrable bovine gamma globulin in his serum. He was one of those Schick tested and the authors suggest the Schick test may have been the stimulating factor in his case.

The authors conclude that absorption from the newborn or adult gastrointestinal tract of bovine antibodies (diphtheria antitoxin) present in milk or serum is virtually non-existent.

Since completion of these studies the authors have carried out similar experiments with bovine colostrum, again with negative results. *Frank Russell*

WEDGWOOD, R. J., with the technical assistance of Gloria SCHWARZ. **Measurement of the Components of Complement by the Reagent Titration Technique.** *Ztschr. f. Immunitätsf. u. Exper. Therap.* 1959, Oct.–Nov., v. 118, Nos. 4/5, 358–67, 4 figs. [16 refs.]

GUSTAFSSON, B. E. & LAURELL, C. B. **Gamma Globulin Production in Germfree Rats after Bacterial Contamination.** *J. Exper. Med.* 1959, Nov. 1, v. 110, No. 5, 675–84, 5 figs. [13 refs.]

"The earlier observed pronounced hypogammaglobulinemia in germfree rats of different ages has been confirmed. Using an immunologic technique the concentration of immunologic gamma globulins was found to vary between 10 and 15 per cent of the values observed in ordinary rats. Upon contamination of germfree rats with the normal microbial flora a pronounced lag phase was noted before the gamma globulin level became normal. This lag phase was most pronounced in growing rats.

"Newborn rats seem to start gamma globulin production more rapidly than older germfree rats. The response with regard to gamma globulin production on contamination of germfree rats with different types of bacterial cells through the natural routes is not identical."

SOUTHAM, C. M., TAI, May & GREENE, E. L. **Studies of Properdin System in Human Cancer Cell Cultures.** *Proc. Soc. Exper. Biol. & Med.* 1959, Oct., v. 102, No. 1, 63–7, 1 fig. [17 refs.]

"1) Neither properdin (up to 20 U/ml) nor absence of detectable properdin (<½ U/ml) had any apparent influence upon growth rate or morphology of human cancer cells HEp# 2 or J-111 in tissue culture, nor upon their ability to propagate on subsequent homotransplantation. 2) Neither properdin nor complement was rapidly metabolized or inactivated by these cells in tissue culture. 3) Addition of properdin to tissue culture medium (in presence or absence of cells) caused a fall in C'1 titer and accelerated the fall of C'2 and C'4."

MARTIN, H., AMANN, R. & JUNGBLUTH, H. Über die Gewinnung properdinbindender Substanzen mit endotoxinartiger Wirkung aus Blutzellen und plasma. **[The Extraction from Blood Cells and Plasma of Properdin Binding Substances]** *Ztschr. f. Immunitätsf. u. Exper. Therap.* 1959, Oct.–Nov., v. 118, Nos. 4/5, 246–61, 2 figs. [54 refs.]

The English summary appended to the paper is as follows:—

"With the help of phenol/water extraction properdin binding substances can be extracted from human erythrocytes, leucocytes and from dried human plasma. The biological activities of these substances are similar to those of bacterial endotoxins produced by the same technique. Whether these 'endogenous endotoxins' have any clinical significance remains to be determined."

BLUM, Livia; PILLEMER, L.; LEPOW, I. H.; PENSKY, J.; WURZ, Leona. **The Properdin System and Immunity XI. Studies on the Interaction of Zymosan with the Properdin System** [BLUM, PILLEMER & LEPOW]. *Ztschr. f. Immunitätsf. u. Exper. Therap.* 1959, Oct.–Nov., v. 118, Nos. 4/5, 313–28. [25 refs.] **XII. Assay, Properties and Partial Purification of a Hydrazine-Sensitive Serum Factor (Factor A) in the Properdin System** [PENSKY, WURZ, PILLEMER & LEPOW]. *Ibid.*, 329–48. [25 refs.] **[XIII. Assay and Properties of a Heat-Labile Serum Factor (Factor B) in the Properdin System** [BLUM, PILLEMER & LEPOW]. *Ibid.*, 349–57.

AMIRAIAN, K., PLESCIA, O. J. & HEIDELBERGER, M. **Aspects of the Immune Hemolytic Reaction. V. Enhancement of Guinea Pig Complement by Carboxypeptidase A.** *Ztschr. f. Immunitätsf. u. Exper. Therap.* 1959, Oct.–Nov., v. 118, Nos. 4/5, 289–301, 4 figs. [10 refs.]

WHITBY, J. L. & ROWLEY, D. **The Role of Macrophages in the Elimination of Bacteria from the Mouse Peritoneum.** *Brit. J. Exper. Path.* 1959, Aug., v. 40, No. 4, 358–70, 10 figs. (2 on pl.) [21 refs.]

Macrophages are abundant in peritoneal washings from the mouse, and the authors have examined the action of macrophages on a number of bacteria by injecting the organisms intraperitoneally into mice, washing out the peritoneal space, and maintaining the macrophages thus obtained in tissue culture. Tissue culture medium number 199 was used both for washing out the peritoneum and maintenance of cells. Counts were made of bacteria in the washings from the peritoneum and in the supernatant fluids of tissue cultures inoculated from the washings.

Escherichia coli introduced intraperitoneally did not appreciably decrease in number after 45 minutes in the peritoneal space and a further 45 minutes in macrophage tissue culture. Organisms of the same strain which had been treated with specific antiserum for 30 minutes before being injected intraperitoneally rapidly decreased in number both *in vivo* and *in vitro*, the fall in numbers corresponding to the amount of phagocytosis by the macrophages. Similar results were obtained with *Pseudomonas pyocyanea, Salmonella typhi-murium* and Friedländer's bacillus [*Klebsiella pneumoniae*], but treatment of *Staphylococcus aureus* with antiserum had no such effect. An avirulent strain of *Salm. typhi-murium* disappeared faster than a virulent strain, when neither was treated with antiserum. In a mouse immunized against *E. coli*, bacteria of the

same strain were rapidly removed from the peritoneal space and later in tissue culture by phagocytosis.

The death rates and the interval between infection and death were compared in mice injected intraperitoneally with untreated *Salm. typhi-murium* and in mice similarly injected with organisms treated with antiserum. The death rate was lower and the intervals until death longer in the mice infected with organisms treated with antiserum. *I. B. R. Duncan*

IPSEN, J. **Differences in Primary and Secondary Immunizability of Inbred Mice Strains.** *J. Immunology.* 1959, Oct., v. 83, No. 4, 448–57, 7 figs.

"Immunizability is defined in terms of amount of a given antigen that is necessary to confer a defined degree of immunity.

"Pure inbred mice strains were found to differ up to 10-fold in respect to primary immunizability, referring to immunity after one dose of tetanus toxoid. The same strains were tested with two doses of toxoid separated by various time intervals. Whatever the time interval, the strains differed in respect to the amount of the first dose necessary to confer immunity, and the order of immunizability found after two doses correlated with the primary immunizability.

"Although the regression of response on the first dose increased with time interval and was independent of strains, the regression of response on the secondary dose was independent of time—the flatter, the better immunizable the strain. Mean responses for the same set of doses showed a regular linear increase over three weeks.

"The results are interpreted in the light of current hypotheses on antibody production. Differences in immunizability are explained as differences in the ease and speed with which cells are first matured into specific antibody production cells whereas the rate of multiplication of these cells bears little or no relation to immunizability."

Miscellaneous

WILSON, G. S. **Faults and Fallacies in Microbiology. The Fourth Marjory Stephenson Memorial Lecture.** *J. General Microbiol.* 1959, Aug., v. 21, No. 1, 1–15. [41 refs.]

"I have always been interested in technique, and I therefore thought it might be worth studying the part played by faulty technique in reaching erroneous conclusions. I found an almost embarrassing richness of material before me, and I have perforce limited myself to a few illustrative examples."

The examples include the mistaken observations on which the Brown and Kirwan opacity tubes were standardized, the technical error which led to the assumption that there were filtrable forms of tubercle bacilli, and that tuberculous bacillaemia was common, and the unfounded supposition that certain viruses could be cultivated in ascitic fluid containing a small piece of fresh, sterile rabbit kidney. Confusion between natural and experimental disease in animals, or between superficially similar diseases in man (as when NOGUCHI mistook leptospirosis for yellow fever), and failure to appreciate the complexity of a situation (as in biological standardization), have also been sources of serious error.

The author, in this lecture, was speaking of microbiology, but his conclusion that "Nearly all the errors of which I have spoken have arisen from imperfect technique and absence of full controls, or from oversimplification through failure to appreciate the complexity of the situation" has a much wider application. One of the essentials for a scientific investigator is training, which should of course include technique, but also much more, and another is the development of a strong critical faculty. *Charles Wilcocks*

GARVEN, H. S. D. **On the Writing of the M.D. Thesis.** *Scottish Med. J.* Glasgow. 1959, July–Aug., v. 4, Nos. 7/8, 386–91. [14 refs.]

The author refers to a Glasgow professor of medicine who was "distressed by the inadequate and often untidy way in which the results of many of the better-designed researches were presented", and he composed the present short paper as a guide to those who write theses, though the need is not confined to them. Editors are well aware of the fact that there is an art in exposition which is by no means always cultivated—or even appreciated—by scientists who take great pride in the elegance of their scientific work. Like all arts, exposition needs care in its development and practice.

The author briefly discusses the virtues of clarity and the difficulty of achieving it. One essential is orderly presentation, and he goes on to give, with comments, a general scheme of order into which many theses would fit; this is now well known and widely used, and applies to papers other than theses. But the more important theme is that scientific writing is difficult and can only be improved by taking pains; the practice of clear exposition is an essential discipline for scientists who wish their work to be read and remembered.

Charles Wilcocks

Book Reviews

HAMILTON, R. A. [Edited by.] **Venture to the Arctic.** 283 pp., numerous illustrations. 1958. Harmondsworth: Penguin Books Ltd., Middx. [5s.]

This book tells the story of the British North Greenland Expedition from the scientist's point of view. Without delving deeply into the scientific research, each science is taken in turn, the problem to be investigated is stated and the method of tackling it described, and when possible the results are discussed. The Expedition had other aims besides scientific research, but they are not discussed in this book. The activities of the Expedition are described only in so far as they have a bearing on the scientific work.

In an introductory chapter C. B. B. BULL outlines what was known about Greenland before the Expedition left Great Britain to set up its base in Dronning Louise Land, and then follows a chapter in which R. A. HAMILTON sets out the background to the Expedition. F. R. BROOKE describes the survey of Dronning Louise Land and Dove Bugt, and R. A. HAMILTON tells of the meteorological work. In three chapters on geophysics, C. B. B. BULL describes seismic investigations on the inland ice and the gravity survey, and discusses the geophysical results. Medical aspects are dealt with by J. P. MASTERTON, and H. E. LEWIS writes on physiology. Then follow three chapters on glaciology by H. LISTER, in the first of which he deals with the balance sheet, or mass balance, in the second with ablation, and in the third with glacial prehistory. Geology is dealt with by J. D. PEACOCK, and geomorphology by P. J. WYLLIE. Finally, R. A. HAMILTON contributes a chapter on the aftermath and money matters.

In his chapter on medical aspects Masterton gives an excellent discussion of the problems of selecting personnel for such an expedition. Some other lessons which should help future ventures were learnt. In the medical examination attention should be paid to the past history of candidates, one should aim for fitness, but physique itself should not be the first consideration. It is imperative that before departure plenty of time should be available for preparation and planning. The people should know a year beforehand that they are going, and for perhaps 3 months immediately before departure their time should be devoted to expedition matters. More mechanical aids should be available for the unloading and carrying of stores. Even a wheelbarrow would be invaluable. Since so much time is spent in the hut, it is essential that the accommodation should be roomy and well designed, and that it should provide individual privacy, improved ventilation and sanitary arrangements, and better doors. In the sick room there must be strong emphasis on good lighting and a comfortable examination couch. In this expedition the food at Base could have been more interesting —tinned products were used too much. The sledging ration was good, but it was monotonous and badly packed. In close quarters the constant hazard of carbon monoxide and petrol fumes makes it essential to have efficient detectors. Fire is a regular risk, and adequate numbers of extinguishers are necessary.

In his section on physiology, Lewis describes observations on the sleeping habits of members of the Expedition. The winter darkness severely disrupted the pattern of sleep. In most members the disruption was marked by naps and interrupted sleep; then in spring when daylight and darkness alternated there was a return to the more usual pattern, but this was again disturbed during the summer period of continuous daylight. When the monthly sleep was totalled there was little difference over the 2 years.

There was a marked seasonal variation in body weights. In the first year there was an increase of weight during the winter and early spring, and this was repeated in the second year, but with less fluctuation. 3 months after the Expedition left Greenland final weighings in the United Kingdom showed a general falling off in body weight, suggesting that the seasonal variations noted were related to the novel environment of the Arctic. For estimating changes in the amount of subcutaneous fat measurements of skin-fold thickness were made. Those measurements corresponded closely with the changes in body weight.

Mouth temperatures showed no striking changes over the seasons. Routine measurements of the basal metabolic rate (BMR) were made, but whereas some workers have reported a raised BMR as a result of exposure to cold, the value found on the Expedition was almost identical with that given for men in Great Britain. Various estimations were made on specimens of blood taken monthly. In general the haematological findings illustrated the constancy with which the body maintains its internal environment, but there was an interesting finding that some of the fatty-acid constituents of the plasma were greatly raised, especially in samples taken in the winter periods.

While at Base the members were living on a diet slightly above the average diet in the United Kingdom *viz.*, 3,900 calories, and the men appeared to be living in energy balance. While sledging, also, the energy intake and expenditure were found to balance, although the men were living on a fat intake of twice the amount they took at Base, and yet analyses of faeces showed that they were excreting a normal amount of fat. When the food was unlimited while sledging the mean daily intake was 4,800 calories.

Altogether the book is a fascinating account of valuable scientific work. It is well produced and well illustrated. *Thomas Bedford*

CHANDRASEKHAR, S. **Infant Mortality in India 1901-55. A Matter of Life and Death.** With a foreword by Philip M. HAUSER, 175 pp., 4 charts. 1959, London: George Allen & Unwin Ltd., Ruskin House, Museum Street. [20s.]

This monograph reports a painstaking and thorough efforts to arrive at the truth regarding infant mortality

in India, its statistics, its causes and possible remedies. The author has to admit that accurate figures do not exist, and that even the degree of error in the published figures cannot be estimated. He therefore wisely discards the search for statistical accuracy. Figures exist, covering over half a century: they have deficiencies, but they make clear two undoubted facts, one that infant mortality in India has fallen considerably in the last 50 years, the other that the infant mortality rate (now given as 120) is still several times greater than that in developed countries. The rate varies greatly among different social groups, in different areas and as between town and country, from the 308 of the Hindus (scheduled castes) to the 72 of the Parsees.

Causes of infant deaths are known with even less accuracy. They include asphyxia, fevers (especially malaria and smallpox), gastro-intestinal intoxication, prematurity and debility. The cause in each individual case is seldom assessed *post mortem* by a doctor, but in the mass these and a few other standard causes account for the vast majority. Behind these lie other basic causes. A high birth rate in itself raises the infant mortality rate, for the later children in a family have lower chances of survival: the older the mother, and the more frequent her pregnancies, the less likely are her children to survive. Children of very young mothers are also highly vulnerable. Birth customs cost many lives, of infants and of mothers—and if the mother dies, who will provide a milk diet for the baby? Weaning methods need radical alteration. The illiterate *dai* must be replaced eventually by a properly trained midwife. Sanitation and fly control are needed, to diminish the loss from gastro-enteritis.

It cannot be said that the combination of high birth and infant mortality rates is a modern version of the survival of the fittest, for the factors which make for high infant mortality seem to be the very same as those which make for poor health and physique in the survivors. There is, therefore, not even a biological excuse for inaction: the rate should be reduced. What is to be done? Improvement of the environment is one obvious need. The decline of mortality in the young age-groups which accompanies the control of malaria is well known. What is not so well known, though to the reviewer it has always seemed most important, is that the establishment of environmental sanitation in England in the second half of the 19th century did not result in any decline of infant and toddler mortality rates, though no doubt it paved the way for the rapid decline that followed the introduction of personal health services.

Summarized, the author's conclusions point, as they must do, to the advancement of standards of living and of education in all its forms. For the first, industrial development must take place. For education, every means available from the university to the mass education campaign with the barely literate teaching the illiterate must be put to the maximum use.

This is an important and valuable study, with a wealth of figures, gathered not only from India, and presented with full appreciation of their individual accuracy and value. *B. B. Waddy*

CRUICKSHANK, R. [M.D., Ch.B., F.R.C.P., D.P.H.] & GLYNN, A. A. [M.B., B.S., M.R.C.P.] [Editors.] **Rheumatic Fever. Epidemiology and Prevention. The Proceedings of a Seminar held at the International Children's Centre, Paris, 25–27 September 1956.** 193 pp., illustrated. 1959. Oxford: Blackwell Scientific Publications Ltd., 24–25, Broad Street. [25s.]

The book is a report of the proceedings of a seminar held at the International Children's Centre, Paris in September, 1956 and forms an authoritative and concise summary of our knowledge of the epidemiology and prevention of rheumatic fever. It is divided into 4 parts and each consists of short contributions by experts in the subject followed by a condensed report of the discussion that took place at the seminar and a list of references. Part I deals with the bacteriology and immunology of group A streptococci, and practical points regarding group and type serology and the titration of the various antibodies are discussed. Part II treats of the epidemiology of haemolytic streptococcal infections with reference for example to carriers and scarlet fever, streptococcal sore throats in children's homes, and the effect of penicillin in rapidly rendering patients suffering from scarlet fever non-infectious. Part III deals with the epidemiology of rheumatic fever. The evidence for the relationship of group A haemolytic streptococci and rheumatic fever is dealt with in some detail as are the titration and significance of antistreptolysin O and other streptococcal antibodies. Factors other than streptococcal infection—heredity, malnutrition, climate and other environmental factors—are all discussed. Part IV deals with the possibility of preventing rheumatic fever. Sulphonamide and penicillin prophylaxis are discussed and tables given of the effectiveness of varying doses of the latter and of its use intramuscularly or orally.

The outstanding features of the book are the conciseness of all the contributions and yet the comprehensiveness of the information. The book is easy to read, it rapidly brings the reader up-to-date in his knowledge of the subject and also acts as a guide to further study. It can be thoroughly recommended to everyone interested in any of the aspects of the rheumatic fever problem. *A. B. Christie*

WORLD HEALTH ORGANIZATION. **Pharmacopoea Internationalis. 1st Edition. Supplement. Recommended Specifications.** pp. xx + 224, numerous charts. 1959. Geneva: Palais des Nations. [Sales agent in U.K., H.M. Stationery Office.] [25s.; $5.00; Sw.fr. 15.–.]

[Review appears also in *Tropical Diseases Bulletin*.]

This Supplement completes the first edition of the International Pharmacopoeia; volumes I and II were published in 1951 and 1955 respectively [see this *Bulletin*, 1952, v. 27, 699 and 1956, v. 31, 600].

Among the 94 new monographs are hormones, antibiotics (tetracycline, erythromycin and polymyxin B), antiseptics (benzalkonium and cetrimonium), contrast media (acetrizoic and iopanic acids) and newer preparations such as ethyl biscoumacetate, chlorcyclizine,

chlorpromazine, hexamethonium, nalorphine and hydrocortisone. There are also a few simple chemicals such as borax, boric acid, potassium nitrate and zinc oxide for which standards have been found to be necessary. The new monographs for substances used in the tropics include chloroquine sulphate, primaquine diphosphate, pyrimethamine, diethylcarbamazine, suramin, diodoquin and *gamma* benzene hexachloride.

The 17 appendices cover the necessary control tests and dosages of the drugs described in the new monographs and bring the list of International Standards up-to-date. In addition, there is a description of a rapid test for alkalinity, lead and arsenic in glass containers for injections, and a section on the determination of *p*H. From a useful set of diagrams in Appendix 7, it is easy to find the amount of sodium chloride which needs to be added to a solution of a drug to give an isotonic solution suitable for injection.

Some amendments to the previous volumes are presented in an annex and there is a comprehensive index to the whole first edition. The format, paper and binding are uniform with volumes I and II.

In the structural formulae which accompany the monographs positive charges have been located on nitrogen atoms in the salts of basic substances. This is satisfactory for the quaternary ammonium compounds but is of doubtful validity for substances containing more than one primary, secondary or tertiary nitrogen atom, because there may be uncertainty regarding the site of protonation. Thus an error has resulted in the instance of diethylcarbamazine citrate, in which the the proton has been assigned to the feebly basic amido-nitrogen atom.

The International Pharmacopoeia is published by the World Health Organization to provide acceptable specifications for medicinal substances and serves as a guide to national authorities in the establishment of legal standards. It is available in English or in French and a Spanish edition is in preparation.

L. G. Goodwin

STABLEFORTH, A. W. [D.Sc., M.R.C.V.S., D.V.S.M.] & GALLOWAY, I. A. [D.Sc., M.R.C.V.S.] [Editors]. **Infectious Diseases of Animals. Diseases due to Bacteria.** Vols. 1 & 2. pp. x + x + 810, illustrated. 1959. New York 3: Academic Press Inc., 111 Fifth Avenue, N.Y. London: Butterworths Scientific Publications, 4 & 5, Bell Yard, W.C.2. [$33.0; £8.]

These two volumes provide the most comprehensive account that is available of the infectious diseases of domestic animals caused by bacteria and fungi and, as such, fulfil a need that has existed for a long time. They form only part of a work, the remaining volumes of which are concerned with diseases of viral and protozoal origin and are to be published later. The relevant literature has been well covered and about 4,500 references are quoted. Apart from their obvious value to veterinary scientists and post-graduate students, these two volumes will be found useful by those engaged in many related fields (including medicine and public health) who now have available, for the first time, an authentic reference book to which they can turn when requiring information on the bacterial diseases of animals.

This work is divided into 20 chapters, each being written by an expert in the subject concerned. Apart from one chapter devoted entirely to diseases caused by fungi, each chapter deals with diseases caused by the members of one bacterial genus. Most of the authors are British but, with a few exceptions, the diseases that are more prevalent or are confined to foreign countries have not been neglected. Diseases are considered from all aspects but special emphasis is placed on the characteristics of causal agents, pathogenesis, epidemiology, prevention and control. Treatment is adequately dealt with as far as specific chemotherapy and serotherapy are concerned.

This work as a whole suffers from a certain lack of balance which is not surprising in view of its many authors. For example, a very full description of the causal agents of the diseases is given by most authors; others provide little more than sufficient information to permit or to aid identification. Again, it is debatable whether the space allocated to the different diseases has been correctly proportioned. The diseases with which *Escherichia coli* are associated include those which produce serious losses in calves, pigs, piglets and chickens. Yet they are all considered in 9 pages. (Even so, it is surprising that no reference is made at all to the chemotherapy of these diseases.) By contrast, the chapter on diseases due to *Brucella sp.*, the largest, encompasses over 100 pages.

The diseases of cattle, sheep, pigs, horses and goats receive full attention, but poultry diseases, in a few notable instances, are neglected. Avian tuberculosis, for example, is dealt with in only a few lines while chronic respiratory disease of poultry (CRD) which is now generally accepted as being caused by pleuropneumonia-like organisms, is not mentioned in the 39 pages allocated to diseases caused by these organisms, despite the fact that CRD is one of the major causes of loss in the broiler industry. Another important disease of broilers, *Escherichia coli* infection, also receives no mention.

Despite these criticisms the two volumes can be thoroughly recommended and deserve a place in the libraries of all institutions directly or indirectly concerned with diseases of animals.

H. Williams Smith

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